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SUMMARIES OF THE RESEARCH SUPPORTED WITH PROJECT GRANT FUNDS BY THE DIVISION OF RADIOLOGICAL HEALTH, SUPPLEMENT NUMBER 1

Paul F. Hahn

Public Health Service
Washington, D. C.

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FOREWORD

The Division of Radiological Health of the Public Health Service is responsible for planning, conducting and coordinating a national program for the evaluation and control of radiation hazards so that the benefits of radiation may be achieved without undue risk. One of the major categories of this national program is the research activities of which the extramural grants program plays an important part.

Research grants allow the utilization of specialized scientific competence available in non-profit organizations and institutions of higher learning. In the Division of Radiological Health a Research Grants Program was instituted shortly after the Division was formed and has held an important position ever since that date.

This compilation has been prepared in order to acquaint scientists in related fields of the types of grants supported under the Research Grants Program and to afford an opportunity for communication between researchers where this is mutually beneficial.

Donald R. Chadwick, M. D.
Chief, Division of Radiological Health

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STATEMENT OF CREDIT

The following abstracts have been contributed by the principal investigators of the projects involved with the understanding that they may be published in the form presented.

No additions, deletions or other editorial changes have been made with the exception of correction of obvious typographical errors.

If further detailed information regarding projects is desired, it is suggested that the investigators be contacted directly.

Paul F. Hahn, Ph. D.
Chief, Research Grants Staff
Division of Radiological Health

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REVIEW OF APPLICATIONS

Applications for Public Health Service research grants are reviewed by study sections or special committees of non-federal consultants expert in their respective scientific and technical fields. These groups were established in order that the advisory councils and committees and the Surgeon General might have the benefit of the best scientific and technical advice on all applications for research grants.

The study section recommendations are submitted to the Advisory Councils or Committees for final review. These latter groups, which are established by Act of Congress, then recommend to the Surgeon General those applications which merit support. The Surgeon General may not approve any research grant that is not favorably recommended by the appropriate council or committee. They also advise on matters relating to programs and activities in their respective areas of research.

In their impartial evaluation of applications, the study sections, committees and councils take into consideration (1) the scientific merit and significance of the project, the competency of the proposed staff in relation to the type of research involved, the feasibility of the project and the likelihood of its producing results; and (2) the adequacy of the applicants' resources available for the project and the amount of grant funds necessary for completion.

The National Advisory Environmental Health Committee serves as the national level advisory body for the Research Grants Staff of the Division of Radiological Health.

PROGRAM AREAS SUPPORTED
BY THE
DIVISION OF RADIOLOGICAL HEALTH
IN ITS
EXTRAMURAL RESEARCH GRANTS PROGRAM

I. Effects of Radiation on Man and Matter

- A. Human Epidemiology Studies
- B. Radiation Physics and Chemistry
- C. Supportive Radiobiological Studies
  1. Genetic Radiation Damage
  2. Somatic Radiation Damage
  3. Aging Due to Radiation Damage
  4. Radiation Damage Mechanisms
  5. Modification of Effects

II. Measurement of Radiation Exposure

- A. Methodology and Instrumentation
- B. Population Exposure

III. Development of Methodology for Exposure Reduction and Control

- A. Studies of Environmental Pathways
- B. Studies of Protective Action in Environmental Contamination
- C. X-ray Exposure and Radionuclides

IV. Others

- A. Radiation Meetings and Conferences
- B. Non-Ionizing Radiation
- C. Beneficial Applications

I. EFFECTS OF RADIATION ON MAN AND MATTER

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RH 00001
RAD

"PHYSIOLOGICAL REACTION OF THE AVIAN EMBRYO TO X-RAYS"

This investigation was established to determine if deviations in physiological responses could be determined in survivors of embryonic irradiation exposure. The avian embryo provides a useful tool for this type of research due to the fact that it develops independently of maternal connection and resulting complications.

Survivors of LD_{50} (full term embryo) exposures administered at 0, 1, 2, 3, 9, 12, 15 and 18 days of development were grown and maintained through the first year of maturity. During the post-hatching maintenance period, these survivors were evaluated for growth rate and growth pattern as well as reproductive capability and reproductive capacity.

Male and female survivors of treatments at 2, 9, 15, or 18 days of development exhibited significant differences in growth rates and patterns at 4, 8, 12, and 16 weeks of age. The trend established by analysis of growth patterns was for a progressive depression in body weights through the twelfth day-treatment; then the depressing effect decreased and body weights increased through the eighteenth day-embryo treatments.

Female survivors of embryonic exposures at 2, 9, 12, 15, or 18 days of development were evaluated for reproductive capacity based upon ovulation rate, as measured through egg production. This investigation of egg production for 224 days delineated that all irradiation survivors had significantly lower ovulation rates than did the non-irradiated controls. The significance of this observation rested in the extremely low ovulation rates of the survivors of irradiation on the twelfth day of development; these same survivor females had demonstrated the severest depression in growth rates.

Male survivors of embryonic irradiation failed to display any significant deviations from control values in their ability to fertilize the gametes of control hens or to subsequently produce viable progeny. Histological examination and measurement of testicular tissue from these survivor males did not indicate any significant volumetric differences in seminiferous tubule development levels.

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Chicks produced from the artificial mating of survivor females with control males did not display any deviations in growth rates or patterns. Female progeny of these matings were found to ovulate at a rate lower than the progeny of non-irradiated individuals.

Exposure to irradiation during embryonic development apparently exerts a depressant effect upon the physiological mechanisms concerned with growth and reproductive capacity. Both male and female survivors demonstrate significantly depressed growth patterns, and female survivors also demonstrated depression of ovulation rate. Male survivors demonstrate no residual impairment in reproductive potential. Matings conducted between survivor individuals, between survivor and control individuals, and between control individuals produced comparable percentages of embryos and viable progeny.

Progeny produced by survivor individuals demonstrated few differences in either growth rates or reproductive capacities. Future investigations are planned to delineate the magnitude of these transmitted physiological disturbances in progeny performance.

***

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RH 00062
GEN

"MUTATION OF POLYGENES IN DROSOPHILA BY IRRADIATION"

Female fecundity of Drosophila melanogaster second chromosome recessive lethal heterozygotes in homozygous and heterozygous genetic background

Eighteen spontaneous lethals were introduced separately to homozygous S-2 genetic background by a system which allowed an average of 13.7% residual heterozygosity from the original lethal stock, i.e., a section averaging 39.4 crossover units surrounding the lethal remained unsubstituted by S-2. Fifty 1/ + females, divided into five replications, were observed for egg production during the third through sixth days after eclosion. Heterozygous background was produced by crossing the S-2 background strains with W-109, and unrelated homozygote.

In homozygous background where the female fecundity (f) of S-2 is taken as 1.000, the mean f of the 18 lethal heterozygotes was 1.475, all values being greater than 1.19. When the f-values were corrected for the 13.7% residual heterozygosity, the mean f became 1.280 and only one of the 18 values fell below 1.0. Five of the corrected f-values were considered to be significantly greater than 1.000, while the group as a whole could be considered to represent samples drawn from a population the over-all mean of which was greater than 1.000 since 17 of the 18 values fell above 1.000.

In heterozygous background, where the hybrid S-2/W-109 was taken as 1.000, the mean f-value was 0.961, all values being less than 1.05, eleven of them less than 1.000. There was no rank order correlation between f-values obtained from homozygous and heterozygous background.

Larval, or pre-adult, viability has been the usual means of estimating the consequences of heterozygosity for deleterious genes, whereas f is equally important in over-all fitness. The present study indicates that the f-consequences are relative to the degree of heterozygosity of the genetic background.

The structure and function of the 55.1 region in the second chromosome of Drosophila melanogaster

Using a system of partially overlapping deficiencies, we have decided that rl and stw, located at 55.1 on the recombination map of the second chromosome, are separated by at least eight functionally distinct genetic elements, with rl on the left and stw on the right of this region which has about 0.05 units of recombination in its total length. The inside elements in this region are all fully complementary recessive lethals. This indicates that the region is organized somewhat differently than has been found in Drosophila before. There appear to be a series of contiguous cistrons all having closely related functions. Lethality, however, is only a crude criterion of function, and we must attempt to decide the nature of the lethal effect of mutants in this region.

X-ray induced polygenetic mutants of modifiers of the heterozygous viability of a recessive lethal

We have established populations in which 1(2)551 is segregating at equilibrium. The equilibrium value is about 40% lethal heterozygotes. We have irradiated the genetic background without irradiating the

RH 00062 (Continued)

second chromosome in which the lethal is segregating, and have studied the lethal frequency levels in the populations for a period of twenty generations following the irradiation. The complete result is not known yet but lethal heterozygote frequencies did not change during the generations immediately following the irradiation. Recent indications have been that the lethal frequency is decreasing in the irradiated populations which means that modifiers of heterozygote viability have been induced. Dose-frequency calculations indicate that the mutation rate of these modifiers is about one hundred times higher than the x-ray induced mutation rate of so-called visible mutations.

***

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RH 00090
PTH

"ENHANCEMENT OF RADIOSTRONTIUM (Sr-90) EXCRETION"

The purpose of the investigative project was to determine the long-term effects of various chelating agents and antimetabolites upon the removal of radiostrontium (Sr-90) from the mammalian body. Strontium-90 accounts for a relatively large fraction of the fission products produced in a nuclear explosion. Strontium-90 has a relatively long radioactive half-life of 27.7 years and decays by beta negatron emission (0.61 mev) to yttrium-90 which also decays by beta negatron emission (2.18 mev) with a half-life of 64 hours to stable zirconium. Radiostrontium is a bone-seeking isotope and significant accumulation in the skeleton induces osteogenic sarcoma in addition to other neoplasms.

Among many studies, for example, a study has been completed of the effects of parenterally administered sodium phytate upon the incidence of strontium-90-induced osteogenic sarcoma. Animals were treated with sodium phytate (250 mg/kg. of body weight) at various intervals before and after administration of a cancerogenic dose of strontium-90 (0.75 uc per gram of body weight). Treatment with sodium phytate was continued for 28 consecutive days following the initial dose. Parenteral

RH 00090 (continued

administration of sodium phytate resulted in no significant reduction of the incidence of osteogenic sarcoma. Pilot studies indicated that oral administration of sodium phytate enhanced excretion of strontium-90, and confirmation of this observation was reported by Uchiyama. Early investigations in our laboratory also demonstrated that rats were able to tolerate considerably higher doses of sodium phytate (950 mg./kg. of body weight) than those utilized by Uchiyama. (Uchiyama, M. Study of the prevention of internal contamination by radioactive strontium. U.S.A.E.C. Document, 1961, AEC-tr-4482.) Significant radiostrontium elimination was achieved employing the oral administration of sodium phytate.

A Study of the roentgenographic development of the radiostrontium (Sr-90) induced osteosarcoma was also completed, the results being published in the Journal of Bone and Joint Surgery, Vol. 47-A, March, 1965

***

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RH 00106
RAD

"GAMMA IRRADIATION OF CYSTEINE"

The reaction of protective agents such as cysteine with free radicals produced upon the irradiation of water is being studied in order to obtain basic information on the reactions. The effects of pH, oxygen, anion and added scavenger upon the 'G' value has been determined. A cobalt 60 source has been used, providing a dose rate of 7×10^{16} e.v./gm/minute. Cysteine concentration has been determined by colorimetric analysis. Products of the irradiation are separated by paper chromatography and thin layer chromatography. Products are identified by comparison of the R_f values with the R_f values of known compounds. Quantitative analysis of the products is done by elution from the chromatogram followed by a colorimetric analysis, or by a development upon the paper followed by a photometric determination for quantitative analysis.

A comparison of the results of aerated solutions with the results of solutions purged with Nitrogen has been made.

RH 00106 (continued)

Typical results obtained to date are given in the following table:

TABLE I

'G' (-cysteine) as a Function of Solution Irradiated
Cysteine Concentration: 1×10^{-3} M, aqueous solution
Dose Rate 7×10^{16} e.v./gm/min

Solvent	pH	G, aerated	G, N_2 purged
0.2 M HCl	0.70	11.5	7.4
0.1 M H_2SO_4	0.95	11.0	7.0
0.2 M HNO_3	0.70	11.8	11.8
1.1×10^{-3} M H_3PO_4	3.0		6.3
5.7×10^{-2} M HOAc	3.0		6.3
0.1×10^{-3} M KBr	0.95		6.3
0.1 M H_2SO_4 +			
1.0×10^{-3} M KBr	0.95		6.3
0.1M H_2SO_4 +			
0.01 M KBr	0.95		6.3

Work with additional radical scavengers is continuing in order to determine the role played by both OH and H radicals. Work is also in progress on the determination of the amounts of H_2S formed in the various solutions, and the effects of the scavengers upon the yield of H_2S.

***

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RH 00197
BBC

"RADIATION AND PROTECTION IN ENZYME SYSTEMS"

The radiosensitivity of many metallo-enzymes may be altered by the addition of both inorganic and organic compounds prior to x-irradiation in aqueous solution. Previous investigations indicate that the degree of radiation response is a function of the radiation induced changes in or near the active site. Investigations are currently in progress with the metallo-enzyme, catalase. This enzyme lends itself well to radiation effect studies, due to the presence of bound ferric iron which is a functional part of the active site.

Experimental evidence has shown that compounds which have the ability to complex with iron atoms of the enzyme exhibit a greater radioprotective ability than those which act primarily as radical scavengers. Further studies are being conducted using glycerol, dimethyl sulfoxide and selenium derivatives. Evidence of complex formation involving the enzyme and the added agent has been demonstrated by shifts in the optical absorption spectra and by changes in electron spin resonance (ESR) patterns.

Recently we have shown that glycerol modifies the radiation response of catalase as well as of lactate dehydrogenase. From these investigations it seems as though a complex occurs between glycerol and the metal ions present in the enzymes. Further evidence for the existance of a metal-glycerol complex was given by investigations with both ESR and nuclear magnetic resonance (NMR) and optical absorption studies. The absorption peak of Fe^{3+} - glycerol solutions was shifted to longer wave length as compared to the Fe^{3+} aqueous solutions. The difference between the two absorption peaks was found to be about 167A^{o}. The wavelength shift and the occurrance of colored iron-glycerol solutions demonstrate complex formation. The absorption peak for copper-glycerol solutions exhibited a maxima at 2550A^{o}, while this peak was not present in the aqueous copper solutions.

The formation of a complex between metal ions and glycerol could also be demonstrated with ESR and NMR measurements. The well-known Mn^{2+} spectrum with its six peaks is observed in aqueous solution. However, Mn^{2+}- glycerol solutions gave a completely different ESR pattern; a single, very broad signal was obtained. The normal Mn^{2+} six line hf spectrum was observed to be superimposed on this broad signal at 7701<.

RH 00197 (continued)

Glycerol itself does not exhibit an ESR Signal. The hf pattern of Cu^{2+} visible in glycerol disappears in water and the iron signal at a higher g- value is expressed only in glycerol.

Biological and conformational alterations in the enzyme as a result of x-irradiation are being studied both by enzymatic assay and by optical absorption and ESR techniques in order to elucidate, in more detail, the mechanism of protection. Optical absorption studies have been made of both irradiated enzymes solutions and enzyme solutions containing the modifying agent. Several investigators have recently demonstrated the radio-protective effect of DMSO on a variety of systems, including animals, spores, and isolated cell systems. From the evidence which has been presented, it seems as though no single explanation adequately describes the mechanism of protection. Further investigations will be conducted. Results from both enzymatic activity as well as optical and ESR studies indicate that a replacement or a partial replacement of the water molecules which are associated with the bound metal ion in the active site region would result in an observable protection effect by a reduction in the probability of free radical interaction with the enzyme itself.

***

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RH 00211
NTN

"ALTERED METABOLISM STUDIES OF I-131 AND SR-90"

The primary objective of this research is the study of basic mechanisms by which (1) the absorption and utilization of the radionuclides I-131 and Sr-90 may be eliminated, (2) the rate of elimination may be increased, and/or (3) the route of elimination may be altered with specific references to the development of practical means of prevention or lessening the contamination of the food supply.

The effect of the administration of carrier iodine prior to and after the administration of radioactive iodine upon the whole body retention of I-131 in calves has been studied. The technique of whole body liquid scintillation counting was employed to follow Iodine-131

retention. Young male calves were dosed with Iodine-131 and the whole body Iodine-131 retention determined with time. The experiment was repeated except stable iodine (750 mg/100 lb. of body weight as KI) was administered for a period of 12 days preceding the administration of Iodine-131. The previous administration of KI decreased the whole body retention of subsequently administered Iodine-131 by approximately one-half.

These experiments were followed by studies of the effect of several levels of carrier iodine on whole body retention of radioiodine. Starting twelve days prior to Iodine-131 administration and continuing for the duration of the experiment, calves received daily either (1) no supplementary iodine, (2) 50 mg. stable iodine, (in the form of KI), (3) 500 mg. stable iodine, or (4) 1000 mg. stable iodine. On the day of iodine-131 administration and on days 4, 7, and 11, retention of Iodine-131 was measured by whole-body scintillation counting techniques. Each increase of dietary stable iodine increased elimination of iodine. Average per cent of the Iodine-131 dose retained for treatments 1, 2, 3, and 4 was: fourth day, 26.7, 21.9, 15.5 and 7.9; seventh day, 14.3, 6.7, 3.4, and 1.8; and eleventh day, 8.4, 1.7, 1.0, and 0.8.

Studies have also been conducted on the effect of carrier iodine, potassium perchlorate, and pilocarpine nitrate on the Iodine-131 content of hen's eggs. White leghorn laying hens were injected intravenously with a single dose of 20 uc each of I-131 (Na I) and received one of three levels of either carrier iodine, potassium perchlorate, or pilocarpine nitrate. A determination of the per cent iodine-131 dose deposited in the whole egg, yoke, and albumin during a 15 day period revealed that carrier iodine at the levels used was effective in decreasing deposition of the radionuclides in eggs (about 40% decrease) while potassium perchlorate was very effective (about 90% decrease) at the levels studies. Pilocarpine nitrate also decreases Iodine-131 deposition in eggs but also effected a decrease in egg production.

***

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RH 00246
RAD

"EFFECTS OF RADIATION ON THE MIGRATION OF SALMONIDS"

I. Radiation Effects on Saltwater Adaptation:

Mechanism(s) for adaptation to salt water are requisite in the developmental pattern of juvenile freshwater salmonids. Current studies have been investigating the effects of acute exposures of x-radiation on these processes with particular interest focused on the osmotic and ionic regulatory system. Recent results indicate that whole body exposures of less than 1000 r were non-lethal to fish which remained in fresh water for a sixty day post-irradiation period. However, when exposed fish were challenged with a saltwater environment on the second day following irradiation, a significant number of deaths occurred (50 percent at 1000r). Most of the mortalities occurred in two distinct time periods; between 11-15th day post-irradiation and 25-35th day post-irradiation. Sites of histopathological damage in saltwater fish were found principally in the extra-renal system with severe damage noted in the gill lamellae. Evidence suggests that "ion secretory cells" of the extra-renal tissue may be involved. Attempts will be made to quantitate the damage to the gill filamental epithelium and to try to establish the degree of radiosensitivity of the "ion secretory cell."

II. Radiation Effects on Migration Behavior:

Spawning Migration: A small population of juvenile salmonids has been irradiated at two dose levels; 100 r and 250 r whole-body exposure. Each exposure group (10,000 fish) has been marked by fin clipping and were released into the Alsea River, Oregon. Returning adults will be captured by a weir trap at the Alsea River Hatchery and comparisons will be made between irradiated and non-irradiated populations as regards the number of returning adults, sexual maturation, organ development and fecundity.

Downstream Migration: Movement toward the sea will be assayed by an "artificial stream" movement device. Comparisons between irradiated and non-irradiated populations as regards the migration disposition urge will be evaluated.

***

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RH 00288
BBC

"E. S. R. STUDIES OF RADIATION DAMAGE IN BIO-MATERIALS"

Grant No. RH 00288 is concerned with quantitative and qualitative E.S.R. studies of free radicals produced in certain series of biological materials by ionizing radiation. We are doing both polycrystalline and single crystal studies, but emphasis is now on the single crystal work.

Our E.S.R. spectrometer is essentially equivalent to the Varian V-4502 with 100 k.c. modulation and rotating cavity, while the magnet has a rotating base.

The irradiated single crystal samples are first mounted in known orientations by x-ray diffraction techniques, and their spectra are studied as a function of the principal values and principal axes (with respect to the molecular axes) of the hyperfine and g tensors. This allows us to identify the number and kind of chemical species of the damage centers.

We use both mixed and pure systems, of varying relative concentration, in order to detect intermolecular interactions, or transfer of damage. We also have the facilities for low temperature irradiation and E.S.R. observation so that secondary radical processes may be examined.

Quantitative measurements on the number of radicals produced are made by comparison with standard samples, such as D.P.P.H. or Mg ++ ions in a host crystal. Radiation doses are measured using a Fricke type dosimeter, absorption spectroscopy, and a known extinction coefficient. This enables us to obtain absolute yields of free radicals under varying conditions, so that comparisons can be made between different samples.

Single crystal E.S.R. studies make it possible to identify both short and long lived kinds of radiation damage to bio-materials, investigate intermolecular interactions in mixtures (of various kinds and concentrations), and to obtain relative and absolute yields, much less ambiguously than in powder sample investigations.

***

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RH 00293
MCHB

"α-ALKYLOXIMINOMERCAPTANS FOR PROTECTION IN RADIOTHERAPY"

Several mechanisms have been proposed for the protective action of certain chemicals against radiation damage in mammals. In the case of mercaptoamines, it is felt that one mechanism of protective action involves the formation of mixed disulfides between the amine-thiol and enzymes bearing thiol groups. The mixed disulfides produced, rather than the free enzymes, can then serve as targets for free radicals generated during radiation. In this way it is assumed that at least 50% of the enzyme can be spared since scission of the disulfide linkage can presumably occur in two ways. In one the enzyme is oxidized and the mercaptan reduced, and in the other the protective moiety is oxidized and the enzyme is reduced to functioning species.

Protective agents such as cysteamine retain activity when the nitrogen atom is acylated or alkylated. In light of this it would seem, then, that alteration of the character of the nitrogen atom of molecules having the atoms arranged N-C-C-S might provide protective agents. In particular, it was thought that α-alkyloximinomercaptans would be interesting for study, the general structure being:

$$R\text{-}\underset{\underset{NOR}{\|}}{C}\text{-}CH_2SH.$$

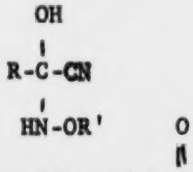
Intermediates in the preparation of the title compounds will be screened routinely. Those to be screened include α-alkyloximinothiolesters, 2-alkyloximinoalcohols, and 2-alkyloximinoisothiouronium salts.

Varying degrees of protection has been observed with many chemicals which bear no relation to mercaptoethylamine. Among these are nitrites, HCN, urethane, ethanol, colchicine as well as several nitriles to name a few. In light of the activity of some nitriles, α-alkyloximinonitriles which can be obtained conveniently by dehydration of the corresponding carboxamides with phosphorus pentoxide also will be screened. The rationale in this case being that the active nitriles function as protective agents through the release of

RH 00293 (continued)

HCN - ideally at a controlled rate. Inspection of the structure

indicates that conceivably attack in the body by water would yield the intermediate



which could decompose to produce HCN and R-C-NHOR'.

Thus far several α-Alkyloximinothioesters and α-Alkyloximino-nitriles have been submitted for testing to the Walter Reed Army Institute of Research. No data on the results are yet available.

***

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RH 00298
RAD

"FOLLOW-UP STUDY OF IRRADIATED TINEA CAPITIS CASES"

The principal facets of this follow-up study of irradiated and non-irradiated tinea capitis cases treated at the NYU Skin and Cancer Clinic include: 1 - location of former clinic patients, 2 - evaluation of their post-clinic illness experience by means of a health questionnaire combined with physician and hospital follow-through for confirmatory diagnoses, 3 - clinical and laboratory examinations of a representative subsample of the irradiated and non-irradiated population, and 4 - dosimetric evaluation of the Adamson-Keinback x-irradiation procedure.

About 1900 cases, each, of irradiated and non-irradiated ringworm cases have so far been processed. Of these cases, 75% - 80% have returned a health questionnaire. The remaining cases include some who are known to be alive but have not yet returned their questionnaires as well as those who have not yet been located.

RH 00298 (continued)

The major finding thus far include 11 cancer cases in the irradiated group and 1 cancer case in the non-irradiated group. There are also 33 documented cases of severe mental illness in the irradiated group and 7 in the non-irradiated group.

Clinical examinations have been done on 605 irradiated and non-irradiated cases. A form of generalized hair thinning was found almost exclusively in the irradiated group. Higher prevalence of balding, greying, benign scalp tumors and subclinical lens opacification was found in the irradiated than the non-irradiated individuals.

Lithium fluroide dosimetry studies with a skull phantom (age 7 years) indicates that the maximum doses to the brain and scalp from the x-ray treatment of tinea capitis are 180 and 800 rads, respectively.

A summary report is being prepared for presentation in the next annual meeting of the American Public Health Association in Chicago (October, 1965). So are detailed reports on the various epidemiologic, clinical and laboratory findings for publications in scientific journals.

***

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RH 00304
RAD

"CELL DIVISION CYCLE AND RADIOSENSITIVITY. METABOLIC STUDIES"

Combined experiments of X irradiation and H^3-thymidine labeling of the chromosomes are under progress to detect when in the cell cycle the chromosomes change their response to radiation from as though single to double. Chinese hamster cells from a quasi-diploid cell line growing in culture are incubated 15 min in a medium containing H^3-thymidine, then thoroughly washed, immediately irradiated with 250 r of X rays and incubated again for various periods of time in a non-radioactive medium containing cold thymidine. At various intervals after irradiation samples of cells are treated with colchicine and hypotonic solution, fixed, stained and autoradiographed. Chromosome aberrations are scored in metaphase.

RH 00304 (continued)

Preliminary experiments showed that a) those chromosomes which had finished (G_2) or were synthesizing DNA (S) at the time of irradiation show aberrations of the chromatid type; b) those chromosomes which had not yet initiated DNA replication at the time of irradiation (early G_1) show aberrations of the chromosome type, and c) those chromosomes which were at the end of G_1 just before DNA synthesis at the time of irradiation exibit both chromatid and chromosome aberrations. The tentative conclusion is then that the chromosomes change their response to radiation at the end of G_1 before the onset of DNA replication. Since the transition between chromosome aberrations to chromatid aberrations probably reflects the time when the chromosomes become double, one should conclude that the longitudinal splitting of the chromosomes occurs before DNA replications.

The pattern of ribonucleic acid and protein synthesis during spermatogenesis in the mouse is under investigation using tritium-autoradiography with H^3-uridine and H^3-amino acids and cytochemical methods, as a basis to understand the differential sensitivity of male germ cells to radiation.

Spermatogonia. The rate of RNA and protein syntheses is much higher in the immature type A spermatogonia than in the mature type B spermatogonia. This difference in the synthetic rates is possibly related to a difference in the degree of DNA condensation between the two types of cells, and might have some implication as to the differential radiosensitivity of the two types of spermatogonia. Nuclear and cytoplasmic protein synthesis occurs at all stages of the division cycle, whereas chromosomal RNA synthesis stops during metaphase and anaphase.

Meiosis. In the autosomes, RNA synthesis ceases or falls to an insignificant level during two periods of the meiotic cycle, early prophase (leptotene to early pachytene) and late diakinesis to metaphases and anaphase I and II. Between these two minima it undergoes a stepwise increase during middle prophase to a peak in middle pachytene, followed by a drop during late pachytene to early diakinesis. In contrast to the autosomes, the heteropycnotic XY bivalent is invariably unlabeled with H^3-uridine throughout meiotic prophase.

Spermiogenesis. RNA synthesis stops shortly after the second meiotic division, in very early spermiogenesis. The ribonucleic acids synthesized during meiosis are completely lost from the nucleus and transferred to the cytoplasm during the course of spermiogenesis, so that middle and late spermatids (from stage 9 to stage 15) do not show any detectable amount of RNA within the nucleus, whereas the cytoplasm exibits fair amounts of RNA that had been synthesized at least a week earlier during meiotic prophase.

RH 00304 (continued)

Histone transition during spermiogenesis. Late or elongated spermatids (stages 11 to 14) are heavily labeled over the nucleus as early as 15 min after injection of H^3-arginine. The nuclear incorporation of H^3-arginine is accompanied by changes in the histochemical properties of nuclear histone (persistance of alkaline fast green stainability after acetylation) which indicate that elongated spermatids synthesize a new nuclear histone very rich in arginine that replaces the previous histone which is a typical histone rich in lysine. A number of evidences suggest that this "arginine-rich" spermatid histone is effectively synthesized within the nucleus, although the alternative interpretation of a cytoplasmic synthesis followed by transfer of the newly synthesized histone into the nucleus cannot be actually rejected.

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RH 00308
RAD

"AUTORADIOGRAPHY OF WHOLE ANIMALS CONTAINING CARBON-14"

Whole Body Autoradiography may provide information that is pertinent to the radiation safety of C-14 compounds in humans; bone is considered the "target organ" for bicarbonate and therefore for many other carbon compounds. Study of placental transfer is another application of the technique.

Two methods of cutting sections for autography were employed. A special microtome housed in a cold room (-20°C) was made available to us through the courtesy of Drs. S. Ullberg and F. Sjostrand, Dept. of Zoology and used to prepare 53 sagittal sections (15 - 100 Microns). More recently a mouse, mounted on dry ice, was cut in cross-section with a band saw into 27 thick slabs (5 mm). Autography revealed striations that can probably be eliminated by trimming the tissue with a microtome. Thin sections were air-dried at -10°C before preparing contact autograms. Thick slabs were autographed at -40 to -60°C without prior drying.

RH 00308 (continued)

In preliminary experiments, 14 mice received C-14 labeled carbonate, acetate, or glucose, 1-2 μc/gm, I.V.; animals were sacrificed at 10 min., one hr. and 24 hrs. In addition, four mice were injected during pregnancy to study the distribution of carbonate or glucose in the fetus and two mice received Alizarin Red S, I.P., to mark the lines of calcification in bone.

Mice that received bicarbonate and sacrificed after ten minutes showed C-14 generally distributed throughout the body with maximum concentration in the liver. High concentrations were noted in spleen, intestines, vertebrae and cranial cavity. Concentrations in muscle and the pleural cavity are much lower. With no-screen x-ray film, the optimum exposure time was 8-20 days. At one hour, 8 day exposures produced autograms that are underdeveloped. Substantial concentration in bone was clearly shown by autograms obtained after 20-64 days. After 23 hours, good autograms can be obtained by contact autography in 17-55 days. The liver retained much of the isotope and high concentrations were noted in the intestine and in the peritoneal lining. One section showed high concentration in an eye. In the sections studied, high concentration was <u>not</u> noted in bone. Animals kept alive for long periods will be included in later studies.

One mouse with glucose - UL was sacrificed after one hour and crossectioned. Autograms (14-28 days) reveal striking concentrations in and around peritoneal linings. This phenomenon will be studied further.

Frozen tissues from pregnant mice are in storage, awaiting autography. The expiration of $C^{14}O_2$ was measured in mice, with the cooperation of Mr. Fred H. Paseman and Dr. E. Wayne Hull of the Biochemistry Department. Each mouse was placed in a gas flow chamber and the expired C-14 measured in a Cary Electrometer Apparatus.

Compound	Exhaled	in ---- hours	Estimated Biological Half-Life
Na Bicarbonate - C14	---	---	8-12 minutes
Na Acetate - 1= C-14	69%	4	2.0 hours
d-Glucose - UL	44%	2.5	2.7 hours
d-Fructose - UL	60%	2.5	2.2 hours
Acetyl salicylic acid-1-C-14	less than 1.7%	3	----

RH 00308 (continued)

Alloxan treated mice ("diabetic")

Compound	Exhaled	in ---- hours	Estimated Biological Half-Life
Na Acetate -1-C-14	50%	4.2	4.2 hours
d-Glucose - UL	19%	2.2	7.5 hours
d-Fructose - UL	37%	2.5	3.8 hours

The frozen carcasses of these mice are available for autography.

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RH 00318
RAD

"GENESIS OF POSTIRRADIATION HYPERLIPEMIA IN THE RABBIT"

Previous investigations of the etiology of postradiation hyperlipemia in the rabbit have established that an enhanced hepatic triglyceride secretion and possible associated increased hepatic synthesis of triglycercides are possible major factors in irradiation lipemia. The present study was conducted to evaluate the role of enhanced formation of liver triglyceride prior to and at the peak of the lipemic episode following whole body ionizing radiation. Adult male and female albino rabbits weighing approximately 2-3 Kg received 1000 r whole body radiation as a single exposure from a 300 KVP GE Maxitron. Dose rate was 106.5r/min calculated at the midpoint of a parrafin phantom. Control rabbits were sham irradiated and all rabbits were prefasted for 16 hours prior to irradiation and throughout the experimental period. At 2, 4, and 24 hours after exposure all rabbits received by intravenous injection 10 μC of palmitic acid 1-C complexd to plasma albumin according to the method of Bragdon and Gordon. Terminal blood and liver samples were removed 30 min after administration of the label and were purified and prepared for analysis by the method of Folch. Plasma and liver triglyceride concentration was estimated by the method of Van Handel and Zilversmit. Radioactivity determinations were

RH 00318 (continued)

performed on liver and plasma triglyceride , isolated by silicic acid chromatography by the method of Filleup and Mead, by liquid scintillation counting.

Similar to the results of others no increase in plasma triglyceride concentration was observed until 24 hours after exposure when plasma triglyceride of the irradiated group were increased a mean 360% above the sham-irradiated group. The amount of label incorporated into the plasma triglyceride fraction of the irradiated group was increased 200% whereas the specific activity ratio was unaltered. At the 2 and 4 hour post-irradiation time intervals plasma triglyceride levels of the irradiated group were similar to those of the sham irradiated control group. However, at the 2 and 4 hour post-irradiation time periods the amount of label incorporated into plasma triglyceride was increased 80% and 100% respectively. The plasma triglyceride specfic activity ratios were increased 40% and 50% at 2 and 4 hours postexposure respectively.

Liver triglyceride concentration was unaltered by exposure to 1000 r at any post irradiation time period. However, at the 2 and 4 hour post exposure time intervals the amount of label incorporated into liver triglyceride was increased 74% and 270% respectively. Similarly liver triglyceride specific activity was increased 123% and 263% above the sham irradiated group at 2 and 4 hours after exposure to 1000 r. By 24 hours the amount of label incorporated in liver triglyceride and the specific activity ratio of hepatic triglyceride of the irradiated group was similar to that of the control group. These data indicate that at the early post-irradiation time periods the synthesis of hepatic triglyceride is increased by exposure to 1000 r and that by 24 hours an irradiation induced synthesis of hepatic triglyceride is no longer evident.

In a collateral experiment we investigated whether irradiation exposure and the subsequent enhanced synthesis of triglyceride was associated with any alteration in the hepatic utilization of the newly formed triglyceride The concentrations of blood ketone bodies at similar post-irradiation time periods was used as an index of the hepatic metabolism of triglycerides. At all of the post-irradiation time intervals there was no alteration in blood ketone concentration between the irradiated and the sham-operated group indicating that the hepatic metabolism of these newly synthesized triglyceride was normal.

RH 00318 (continued)

The results of these studies indicate that irradiation-induced triglyceride synthesis is a major factor in the genesis of post-irradiation lipemia. However, since this synthesis occurs mainly during the early post-irradiation time intervals, the continued elevation of plasma triglycerides must be due to a failure of some mechanism responsible for the intravascular removal of triglycerides.

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RH 00319
RAD

"PROTECTION AGAINST RADIATION EFFECTS ON RENAL FUNCTION"

Renal damage is a frequent consequence of abdominal X-irradiation. This situation imposes severe constraints upon the amount of therapeutic radiation which can be safely delivered to the abdomen. Information regarding radiation nephropathy is scant and mainly morphologic. It is the objective of this study to determine the sequential changes in renal function induced by such irradiation, and further, to observe the effects of pre-treatment with sulfhydryl radiation protective agents on renal function.

Renal function in rats will be estimated by standard techniques, employing an infusion of sodium p-aminohippurate and inulin and collecting urine at timed intervals by means of an indwelling urethral catheter or cystostomy. The animals will be studied in the anesthetized state. If possible I^{131} labelled inulin and iodo hippurate will be used in an attempt to reduce the analytical error incurred in using small biological samples.

The kidney will be irradiated with doses on the order of 1000 to 4000 rads in order to produce kidney damage. As stated above, the renal blood flow and glomerular filtration rate will be determined at appropriate intervals pre- and post-irradiation. Dose-effect curves relative to these parameters will be determined. In addition, the irradiated kidney will be examined histologically, at various intervals and correlated with the foregoing.

RH 00319 (continued)

The radiation protective agents to be evaluated are:

1. 2-Mercaptoethyl guanidine (MEG)

2. Bis (2-guanidoethyl disulfide) (GED)

Lastly, the distribution and metabolism of the protective agents in rats will be studied using S^{35} labelled compound, liquid scintillation counting and paper chromatography and electro phoresis. Special attention will be given to renal concentration of protective agents and attempts will be made to increase the renal concentration without increasing the protective agent concentration in other tissues.

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RH 00321
RAD

"STUDIES IN RADIATION PROTECTION"

The protective action of certain compounds against the effects of ionizing radiation has been the subject of much study. Many problems remain, however, regarding the varying sensitivity of biological systems to radiation damage and the mechanism by which different compounds protect against this damage. Unfortunately, biological systems are much too complex for a definitive study of the molecular reactions which occur in species exposed to radiation. The most meaningful approach to this problem from the point of mechanism elucidation is to study the effects of radiation on relatively simple compounds which have properties in common with biologically important compounds.

Since the major component in living matter is water, most of the initial energy absorption is used in the radiolysis of water,

$$H_2O \xrightarrow{\wedge\!\!\sim} H^{\bullet},\ {}^{\bullet}OH,\ H_2O^{+},\ e_{aq}^{-} \qquad (1)$$

It seems logical that most radiation damage occurs as a result of reaction of these fragments or their products. In this research,

RH 00324 (continued)

we have chosen to approach the problem form the viewpoint that free radicals are likely precursors to the damage. A photochemical method of producing methyl radicals in aqueous solution has been developed. This method allows the evaluation of specific rate constants for H-atom abstractions (Reaction 2) in terms of the completing

$$CH_3 + RH \rightarrow CH_4 + R \qquad (2)$$

Reaction 3. This method makes use of the similarity of the

$$CH_3 + CH_3 \rightarrow C_2H_6 \qquad (3)$$

methyl radical to the H-atom and •OH radical and also the ease with which its reaction products may be analyzed. Measurements are presently underway which will indicate the reactivity of this radical with amino acids, radiation protectors, and radiation sensitizers.

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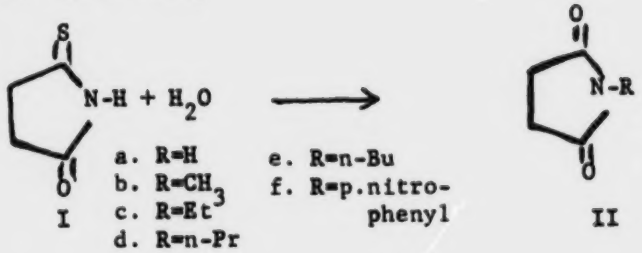
RH 00324
MCHB

"KINETICS OF HYDROLYSIS OF MONOTHIONSUCCINIMIDES"

Preliminary investigations by Dr. J. Doull of the United States Air Force Radiation Laboratory of the University of Chicago indicate that monothiosuccinimides exhibit radiation protection activity. These data prompted the investigation of the influence of N-substitution on stability of the C=S group. Monothionsuccinimides (1) readily undergo hydrolysis to the corresponding imide (II) with evolution of hydrogen sulfide. To date no systematic studies concerned with the effect of substituents in thionimide or thionamide derivatives upon the relative ease of hydrolysis under acidic, basic or neutral conditions have been reported. Information gained in this study will be of value in designing sufficiently stable thionimides for biological evaluation.

The relative pseudo - first order rates of hydrolysis of a series of monothionsuccinimides to succinimides have been investigated.

RH 00324 (continued)



The thionimides were dissolved in 10% ethanol in water ($70.0^{+}_{-}.1^{\circ}$) and the rates of hydrolysis were observed by noting the decrease in absorbancy at the high intensity $\pi \rightarrow \pi^*$ transition at approximately 270 mu to the C=S function. Since hydrogen sulfide is an extremely weak acid ($K_1 = 5.7 \times 10^{-8}$) and since the amount generated is extremely small under the conditions of the hydrolysis its influence upon the reaction rates should be negligivle. Neutral conditions were employed in these preliminary studies since alkaline or acidic conditions would severely enhance the rate. Further, under neutral conditions opening of the ring does not represent a complicating factor. We as well as Cremlyn [J. Chem. Soc., 5547 (1961)] have shown that a quantitative yield of the corresponding imide is obtained when an ethanol-water solution of the thionimide is warmed or evaporated to dryness.

The data which we obtained during these studies can readily be explained qualitatively if one assumes that the rate determining step involves nucleophilic attack of water at the partially positive carbon of the C=S group. Introduction of either alkyl or aryl groups on the imide nitrogen generally slow the rate; a feflection of steric hindrance. However, N-aryl substituted compounds always hydrolyze faster than N-alkyl substituted compounds and this reflects the influence of electronic factors.

The instability of the C=S group is readily observed by noting that the unsubstituted monothionsuccinimide 1a undergoes very facile hydrolysis with a half life of 1.08 hours. Introduction of alkyl substituents on the imide nitrogen increases the stability up to n-butyl (1e) which has a half life of 12.46 hours. A plot of 1/k v.s. the number of carbon atoms in the aliphatic chain indicates that no substantial increase in stability will be realized by extension of the chain length beyond C_4. Interestingly, an 8.2 fold decrease in rate is observed in going from N-H (1a) to N-Et (1c) while only a 1.4 fold decrease in rate is observed in going from N-Et (1c) to N-n-butyl (1e). These data are in agreement with the general prediction

RH 00324 (continued)

which would be anticipated by Newmans "rule of six", and can readily be explained in terms of steric effects where the alkyl substituent slows nucleophilic attack by water in the rate determining step.

Comparison of the rates of hydrolysis of N-aklylmonothionsuccinimides (5 were prepared) with N-arylmonothionsuccinimides indicates that all aromatic compounds (7 were prepared) undergo more rapid hydrolysis than all aliphatic systems. In fact, introduction of a p-nitro group (1f) into the aromatic ring accelerates the rate of hydrolysis to the extent that the half life (0.91 hours) is less than the unsubstituted monothionsuccinimide. In addition, a reasonably good correlation with Hammett's σ-values was obtained ($\rho = +.348$) providing σ for N-p-nitrophenyl (1f) was employed in the plot. These data are rationalized in terms of electronic effects and will be discussed in forth coming publication.

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RH 00325
RAD

"DIFFERENTIAL RESISTANCE OF CELLS TO IRRADIATION"

Comprehensive observations have been made of abnormal motility exhibited by developing sea urchins (Arbacia) following 2537 Å ultraviolet or gamma treatment of sperm alone, eggs alone, or zygotes, the zygotes being irradiated at early non-motile stages from fertilization to hatching and at later motile stages from blastula to young pluteus. Significant differences and resemblances in motility abnormalities were noted. Analysis of cinephotomicrographic records revealed that such differences and resemblances were correlated with the type and strength of irradiation, the three kinds of material irradiated, and, for zygotes the stage of development when irradiated.

Gamma (X-ray_ irradiation is ionizing, deeply penetrating, and non-selective. UV irradiation is non-ionizing, poorly penetrating, and very selective. Both types probably alter DNA, thus inducing developmental abnormalities.

RH 00325 (continued)

Following unilateral UV irradiation of eggs alone, or of non-motile zygotes, interesting progeny resulted. Many individuals exhibited either abortive or complete autotomy accompanied by conspicuous deviations in motility. Such autotomy and such deviations were markedly different from those induced by unilateral UV irradiation of motile zygotes. The difference was correlated with one-sided exposure in the first case and exposure from all sides in the second. Gamma irradiation of eggs alone, or of non-motile zygotes, induced motility deviations unlike those that followed similar UV irradiation.

UV irradiation of sperm alone induced developmental abnormalities essentially like those induced by gamma irradiation of sperm alone. The small size and movement of the sperm cell rendered its DNA content vulnerable to alteration by poorly penetrating UV rays, as well as deeply penetrating gamma rays. A frequent early site for sloughing of injured cells in developing embryos was at the blastopore; a later site was near the apex of the young pluteus.

Certain differences in motility deviations, observed in embryos arising from irradiated sperm alone, compared with those from irradiated eggs or zygotes, were correlated with differences in the cytoplasmic complex irradiated.

Experiments similar to those listed above have also been started with the use of radiomimetic chemical compounds instead of radiation. One of these is colchicine. Another is caffeine.

Colchicine is known to affect cell mitoses in general. It has been used on sea urchin eggs and zygotes. It probably exerts its action principally on astral fibers, thus interfering with the normal separation of chromosomes.

Caffeine is known to delay cleavage of sea urchin eggs. The mechanism by which this is accomplished is thought to be different from the mechanism by which irradiation of eggs causes similar delay.

Additional experiments have now been carried out involving colchicine treatments of (1) sperm alone, (2) eggs alone, and (3) zygotes at early non-motile and later motile stages. Results have been recorded by cinephotomicrography. Detailed analysis of the motion picture films is in progress. Similar investigations with caffeine treatments have also been begun.

The results to date indicate that significant information is being obtained from these comparative studies which may throw light on some basic cellular mechanisms.

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RH 00326
RAD

"A STUDY OF THE THRESHOLD DOSE OF X-RAYS"

When pooled plasma from rats exposed to whole body X-irradiation is injected into the air sac of 15-day old fertilized chicken eggs along with 2.5 microcuries of radioactive iodine-131, a significant increase in the uptake of iodine-131 by the thyroid results. Evidence obtained to date, to explain this finding suggests that the secretion of thyrotropin is increased post-radiation in the exposed animals. This effect has been noted with plasma from rats exposed to doses from 600r down to 200r. The present study is concerned with establishing the threshold dose for this phenomenon with the eventual idea in mind of studying the usefulness of the phenomenon as a biologic determinant of exposure to ionizing radiation.

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RH 00328
GEN

"ANCESTRAL VS. DIRECT EFFECTS OF IRRADIATION ON MAMMALS"

The biological costs in terms of reproductivities were assessed for mice irradiated continuously (22 hours per day on the average) by gamma rays from cobalt-60. Mice exposed to irradiation in the physiological ranges where reproduction is possible, maintained their reproductivities at daily radiation dosages of several thousand times the continuous irradiations received from natural sources.

The gamma radiation was given at eight major dose levels throughout the lifetimes of the mice and those of their descendants, in radiation-charged space carrying 0.6 to 2.7r per day. If irradiation continued above these levels sterility occurred in those mice receiving more than 4r per day over a period of time. First litter productivities of the irradiated mice do not differ from those of a corresponding sample of the same inbred strains not under man-made irradiation

exposure. Sex ratios of the irradiated progenies do not differ in successive generations from those which were unexposed. Early survival and reproductivity of the inbred strains under irradiation proved more dependent on good biological characteristics such as fertility, litter size, milk supply and mothering ability than on ability to resist large amounts of acute single dosages of whole-body irradiation.

Ancestral and direct continuing irradiation effects were determined on ten strains of mice. Five of the ten strains failed to survive beyond the fifth generation. The other five strains have now gone beyond the 12th and one has reached the 28th generation.

Strains differed in their mean life durations, the amount of variation encompassed being 38 percent. For three strains, the life shortening was curvilinear asymptoting to a constant value for each strain. Over-all strain effects were the major contributors to differences in lifespan followed by generation effects. Not all strains reacted similarly to the generations of continuing irradiation.

Correlating ancestral irradiation dosages, within irradiations showed small average values of either sign. The largest value was -0.085 which would account for less than 8/1000 of the variation. The theory that ancestral irradiations, received at rates within the animal's physiological ability to repair, lead to cumulative deterimental mutations in future progenies is heavily discounted by this evidence.

For the rate of irradiation occurring directly to the embryo from conception to birth, or from birth to weaning, the average correlations of dose rates with lifespans were small, -0.06 and -0.006, and statistically insignificant. Correlations of rate dosages under 500r for 38 days after weaning with lifespan were likewise small, -0.06, accounting for about 4/1000 of the variation. They were only of borderline statistical significance. However, the correlations of rate dosages, averaging above 1100r in the weaning to 38 days post-weaning period, were distinctly higher, averaging -0.27. They accounted for about 73/1000 of the variation in duration of life, and the effects were in the highly significant range.

The point of break-over of the continuing irradiation effects is in the late adolescent stage of life, between 500 and 1100r. The threshold change in irradiation effects furnishes another instance of a change in the system of receptors for the radiant energy.

RH 00328 (continued)

The severity of the effects of continuing low-rate irradiations accumulated to the same or larger total dosages and compared with higher-rate acute dosages was shown to be noticeably less. The comparisons were made on the same physiological traits and strains, the radiation being generated by the same machines and laboratory conditions.

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RH 00330
HED

"LOW LEVEL GAMMA RADIATION AND PHYSIOLOGICAL PERFORMANCE"

The principal objective of this research is to investigate the effects of relatively low level gamma radiation on the physiological performance of Spanish goats. To this end three criteria of physiological performance will be evaluated, i.e., reproductive capacity, gross physical performance, and physiological measures of selected systems.

The radiation being used is gamma radiation from a Co^{60} source. The dose rates being employed are 40, 20, 5, 2, and 0 R per 20 hour day. Each dose group contains 10 males and 10 females sexually mature Spanish goats approximately 7 months of age at the beginning of the irradiation periods.

Fertility will be investigated by initially penning the males and females together. If the reproductive performance of any group drops below one half of the control level, the sexes will be separated and controlled matings made to determine fertility of individuals. The females will be bred to nonirradiated males of known fertility; the same males will be used for all dose levels that require use of outside males. Testing of the irradiated males will be on a more limited basis.

Semen will be collected periodically by electroejaculation and analyzed as follows: sperm concentration, semen volume, total number

RH 00330 (continued)

of sperm ejaculated, sperm motility, percent sperm alive, percent sperm normal, and type of abnormalities, and other tests such as oxygen utilization and fructose utilization as necessary.

Performance testing will consist of two types: gross and physiological. The gross testing will consist of the evaluation of the individual animal's ability to perform on a standard obstacle course. Physiological observations will include electroencephalograms, electrocardiograms, and respiration rates as well as routine hematology.

Clinical observations will be made on each animal at frequent intervals to correlate physiological and gross performance observation.

Selected groups of offspring arising from irradiated animal matings will be retained and their growth rate, general performance and reproductive capacity evaluated.

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RH 00334
RAD

"EFFECT OF RADIATION ON AMINO ACID TRANSPORT"

The objective of this work is to study the effects of ionizing radiation on transport of amino acids and small peptides through the cellular membrane of Lactobacillus casei 7469. It is planned to correlate the results with effects on membrane adenosine triphosphatase activity and on protein and amino acid biosynthesis and cell curvival. A similar study involving amino acid transport in Ehrlich ascites carcinoma cells is planned.

At present the main effort is devoted to characterizing the transport systems for neutral amino acids and their peptides in L. Casei 7469. Peptides containing the carbon-14 labeled unnatural amino acids, alpha-amino-isobutyric acid and cycloleucine are being synthesized for use as transport models. A series of tripeptides designed to elucidate stereo-specificity of membrane transport mediators is in use; the synthesis of related tetra- and penta peptides is planned.

RH 00334 (continued)

Earlier work in this laboratory points toward the existence of at least two mediators of entry of neutral tripeptides into L. Casei 4769 (1 2). Transport of glycyl-alpha-aminoisobutyryl-L-alanine, but not of glycylcycloleucyl-L-alanine or glycyl-cycloleucyl-L-Valine, is inhibited by glycyl-L-alanine, L-alanylglycine and glycylglycylglycine in the external fluid. Glycine competes effectively for transport with alpha-aminoisobutyric acid, but not with any of the three tripeptides tested.

It is possible that the peptides of cycloleucine may enter by a mediation similar to the L- or leucine-preferring site for amino acid transport found by Oxender and Christensen (3) in the Ehrlich cell. Affinity for the L-site is strongly affected by stereochemical factors; it increases with increasing lipophilicity of the amino acid side chain and thus appears to be closely related to the lipid nature of the membrane.

The mediator for entry of the tripeptide of alpha-aminoisobutyric acid, however, may resemble the A- or alanine-preferring site of Oxender and Christensen (3), designed to facilitate the entry of substrates less sterically-favored than those which enter readily by means of the L-site. The A-type site should be more sensitive to deficiency of metabolic energy and very probably involves an enzymatic system.

The effect of ionizing radiation on these two peptide transport systems should be quite different. Interference with transport by the A-system would be expected to be brought about by enzyme inactivation and/or uncoupling of oxidative phosphorylation while effects on the L-system would be expected to be related more closely to alterations in membrane structure. A similar dichotamy would be expected for amino acid transport.

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RH 00336
RAD

"EFFECTS OF IRRADIATION OF THE EXTERIORIZED RAT KIDNEY"

This study was designed to provide information relating to the sequence of morphologic alterations which follows the exposure of renal tissue to ionizing radiation. Unilaterally exteriorized kidneys of 2-month-old or 12-month-old male Sprague-Dawley rats were either sham-irradiated or treated with 500 r or 1500 r of 280 KVP X-rays with a HVL of 1.3 mm Cu. Following irradiation, equal numbers of rats from each age and dose group were sacrificed at monthly intervals through 8 months. Techniques used to study the development of morphologic alterations in irradiated and contralateral non-irradiated kidneys included: Histologic section with routine and special staining, gross - and microangiography, and monthly blood pressure measurements.

A difference in the severity of arteriolonephrosclerotic lesions and the rate of their development was apparent when irradiated kidneys from rats of two different ages were compared. The difference was most evident in the 1500 r group where the irradiated kidneys of young rats exhibited extreme morphologic changes, beginning about 5 months after irradiation. The principal alteration which led to a shrunken and fibrotic kidney was a narrowing of arteriolar lumens by a proliferation and/or swelling of endothelial cells. Later, hyaline degeneration and fibrosis of the medial layer of small vessels along with the endothelial cell changes combined to produce varying degrees of occlusion of arterioles and small capillaries. Secondary alterations of glomeruli and tubular epithelium and an increase in interstitial connective tissue resulted from the progressively developing cortical ischemia.

Irradiated kidneys from older rats at the 1500 r level exhibited only mild to moderate changes at the end of the study. This age dependent response appeared to be related to the nature of the vascular alteration. The lumens of arterioles in kidneys from this age group became narrowed primarily by more slowly developing degenerative changes in the medial layer of these vessels with but minimal contribution by proliferation and/or swelling of endothelial cells.

A difference in degree of morphologic alteration was also apparent between age groups at the 500 r level. The arteriolosclerotic changes at this level were of the same nature but were less severe than those

RH 00336 (continued)

seen in kidneys exposed to 1500 r. Contralateral non-irradiated kidneys exhibited hypertrophy of glomeruli and tubules. Minimal arteriolar changes were found in these kidneys unless hypertension developed. With the development of hypertension, degenerative changes became more apparent in all components of the untreated kidney with passing time.

The extreme morphologic changes seen in the kidneys of young rats in the 1500 r group were accompanied by an elevation of systolic blood pressure with group means becoming significantly elevated 7 months after irradiation. Blood pressures of rats in both age groups whose kidneys were exposed to 500 r and the older rats which were exposed to 1500 r remained within normal limits throughout the study.

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RH 00340
RAD

"REACTIVE LIFETIME STUDIES IN IRRADIATED CELLS"

It has been well established that the oxygen effect, a phenomenon of paramount importance in radiation biology, is present during the irradiation of many microorganisms and in mammalian cells in tissue culture. The basic mechanisms through which oxygen acts in modifying cellular radiosensitivity are by no means fully understood. It is strongly suspected that the immediate oxygen effect is due to interaction between oxygen and extremely short-lived radiation-induced species within the cell. Previous investigation of oxygen dependent damage during irradiation of bacteria established experimentally an upper limit of 10^{-2} second for the reactive lifetime of the radiation-induced species. Theoretical estimates of the reactive lifetimes of these species indicate that they may be very much shorter than this time, perhaps as short as 10^{-4} second or even 10^{-6} second.

One specific aim of this project is to study the kinetics of disappearance in irradiated cells of the oxygen dependent radiation-induced species by obtaining new experimental information on the lifetime of

the species. It is hoped that such investigation will lead to further insight into some of the chemical mechanisms of radiation injury in cells, such as an understanding of the mechanism of the oxygen effect.

Another specific aim of this project is to obtain experimental information on the lifetime of the reactive species produced in the irradiation of the DNA present in bacteriophage. Present evidence suggests that the DNA in living cells is the site of most of the lethal injuries produced when cells are irradiated by ionizing radiation. In bacteriophage, which is about 50% DNA, it is almost certain that DNA is the major site for lethal damage from irradiation. It has been shown experimentally that sulfhydryl compounds, when present during irradiation, can afford protection to bacteriophage over an irradiation carried out in anoxia. An hypothesis has been proposed that such protection is the result of reaction between short-lived radiation-induced species in the DNA and a sulfhydryl compound. It is expected that determination of the lifetimes of these species will aid in understanding the basic mechanisms of biological injury at the cellular level.

Methods are presently being developed which will make it possible to study radiation injury in bacterial cells or in their viruses by achieving a gas transfer within 10^{-3} to 10^{-4} second after a pulsed irradiation of comparably negligible duration. The failure to find a change in radiosensitivity when the cells or viruses are introduced into the second gas immediately after irradiation in the first gas establishes an upper limit to the lifetime of the species involved, providing that diffusion into the cells or particles by the second gas is shorter than the time resolution of the experiment. Experimental times as short as 10^{-3} to 10^{-4} second are being achieved through the use of a pulsed high intensity electron source operating on the field emission principle, and through an improved method of achieving rapid gas exchange.

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RH 00347
RAD

"RADIATION EFFECTS OF THYMIDINE-TRITIUM"

Our electron microscope efforts are directed along the following lines:

1. Tracer studies of the interphase cell exposed to labeled nucleic acid precursors (thymidine-H3, uridine-H3, and cytidine-H3).

2. Studies of the nuclear membrane and related nucleoplasm and cytoplasm.

3. Studies of micronuclei resulting from treatment with thymidine-H3.

4. Studies of moribund cells in onion seedling root tip meristems treated with thymidine-H3.

Autoradiography in the electron microscope is supplemented by autoradiography with light microscopy.

The interphase cells which we are observing are those of the onion seedling root tip meristem. We find that radiouridine and cytidine are localized in the chromatin and nucleolus during short treatment periods and this is interpreted to be the site of RNA and RNA synthesis. Littau et al.[1] have shown that isolated nuclei from calf thymus incorporate uridine-H3 so that label appears in the diffuse (rather than electron dense) portion of the nucleus. This they construe to be where active DNA is making messenger RNA. Thus it appears that in the undifferentiated interphase cell of the onion root tip meristem (unlike the isolated nucleus of calf thymus), RNA syathesis is carried out along the chromatin thread itself.

Electron micrographs of interphase nuclei fixed with $KMnO_4$ show the relationship of chromatin and the less dense nucleoplasm to the envelope with its pores better than those of cells prepared otherwise. We are studying electron microscope autoradiographs in an effort to detect movement of material through pores or discontinuities in the nuclear envelope.

RH 00347 (continued)

We have observed micronuclei in ordinary electron micrographs as well as in autoradiographs. It seems clear that some micronuclei too small to be seen with the light microscope are produced with thymidine-H3 treatment. We are also trying to establish the membrane relationships of micronuclei in the cytoplasm.

In an earlier phase of this work we have gathered information on gross changes in cells following massive irradiation with thymidine-H3. We are currently trying to study such cells with the electron microscope.

(1) V.C. Littau et al.: Active and inactive regions of nuclear chromation as revealed by electron microscope autoradiography. Proc. Nat. Acad. Sci. 52:93 (1964)

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RH 00358
RAD

"EFFECTS OF HIGH ENERGY RADIATION ON LIPIDS"

The initial effect of high energy radiations on lipids is to form free radicals which can react further in many ways with each other or with other components of the irradiated medium. If oxygen is present the free radicals react rapidly with it to form peroxide free radicals. With unsaturated fats these free radicals start an oxidative chain mechanism similar to that prevailing in autoxidation. The location of these free radicals along the fatty acid chain, however, has not been determined.

The free radicals formed in irradiated fats and their primary reaction products can react further with other components of fat-containing substrates such as foods and other biological tissues. The extent and possible significance of these secondary reactions are not well known.

In addition, many other compounds such as carbonyls, fatty acids and hydrocarbons of various chain lengths have been detected in

irradiated fats, but the exact nature of these compounds and the effects of various conditions of irradiation and post-irradiation storage on their formation have not been adequately studied.

The aims of this project are:

1). To study the products formed during irradiation of various lipids.

2). To study the effect of irradiation of the physical and chemical properties of phospholipids, lipoproteins and other complex lipids.

3). To study the effect of irradiation conditions and of pre- and post-irradiation treatments on these changes.

4). To determine what effects these irradiation products and other changes have on essential constituents of biological tissues and on normal biological reactions.

5). To relate these changes to the problems associated with the use of high energy radiations for preservation of foods, for radiotherapy and in the treatment of radiation injury.

This project has only recently been initiated and the first phase of the study has just started. So far, efforts have been concentrated on devising means for reproducible, controlled irradiation of a large number of samples under various conditions and for handling irradiated materials to recover irradiation products with minimum additional changes or losses.

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RH 00367
RAD

"BIOLOGICAL MECHANISMS OF RADIATION DAMAGE"

The objective of this program is to identify biological mechanisms underlying a series of the classical signs of radiation sickness observed in the rat and other higher animals following exposure to ionizing radiation.

After a near-lethal dose of x-rays or cobalt gamma rays, the rat stops eating and loses weight. It was initially thought that this weight loss was a simple reflection of the decreased intake of food. This is not true. A major portion of the weight loss in the partially starved (pair-fed) animal is due to a decrease in gastro-intestinal contents, body fat and liver weights. In the irradiated rat, the weight loss is due to a decrease in muscle mass (which begins within a few minutes after irradiation). Tissue weight studies have been extended to other conditions of radiation exposure including large doses to the head, thorax, or abdomen. In all cases there is a loss of weight in the radiosensitive tissues even though these tissues were in a shielded area. For example, there is a marked loss in weight of spleen, testes, and small intestine, when only the head was exposed to radiation. Parabiosis provides a high degree of protection. Current work on tissue damage includes studies of the incorporation of tritium labelled thymidine and C_{14}-tagged orotic acid into tissue DNA. Data suggest that tissue destruction is due in large measure to some factor other than starvation or direct action of radiation upon tissue.

Radiation sickness is characterized by a marked upset in fluid and electrolyte balance in all species. The electrolyte balance has been studied on the rat following exposure to a mid-lethal dose of x-rays. Balance studies have been carried out both on the whole rat and on each of the different tissues of the body. Current work on tissue fluid balances should allow a reevaluation of the electrolyte data in terms of intercellular electrolyte concentration changes. These will be correlated with functional changes occuring in different portions of the body (heart and liver in particular). In the case of the heart, work is proceeding on the electrocardiogram, heart sounds and roentgenographic heart size as measures of functional change.

RH 00367 (continued)

Results thus far suggest that some generalized mechanism(s) of radiation damage exists, either hormonal or nervous, that controls tissue DNA synthesis, tissue weight loss, and certain aspects of electrocardiographic change. Work is directed toward a more complete understanding of this generalized control mechanism. The more precisely measurable of the above biochemical and physiological effects will be used as indices of radiation damage in the different portions of the body. Such techniques as parabiosis, nerve section, and drug response will be used as procedures for distinguishing between nervous and humoral mechanisms.

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RH 00373
NTN
(formerly
RH 00257)

"CHARACTERIZATION OF RADIOPROTECTIVE FACTOR IN ALFALFA"

A substance present in green plant foods (such as alfalfa and broccoli) reduces the radiosensitivity of guinea pigs fed a basal diet of oats and wheat bran. There is no apparent requirement for the factor in the presence of a nutritionally adequate, semi-synthetic diet. The total effect of the plant substance cannot be duplicated by addition of known essential nutrients to the cereal diet, although partial reduction in radiosensitivity is achieved by providing adequate levels of vitamins and crude steamed bone meal. Increasing the protein level of the diet by addition of casein is detrimental in the absence of a mineral supplement and has no effect on radiosensitivity in the presence of added mineral mixture, in spite of improved growth.

A lyophilized fraction of alfalfa prepared by extraction with water at room temperature is as effective in decreasing radiosensitivity as is alfalfa meal or fresh alfalfa. The effective agent in the cold water extract is insoluble in hexane and in ethanol. No radioprotective activity is retained in extracts prepared with hot water, in the water-insoluble residue or in ash. Activity was lost during dialysis of water extract and reduced by autoclaving whole alfalfa meal. Isolation from the cold-water-extract is continuing.

RH 00373 (continued)

In an attempt further to elucidate the mechanism of action of the alfalfa substance(s), the microflora of the guinea pig cecum have been examined. In the presence of alfalfa, cecal population of E. coli was reduced and B. subtilis was found in abundance. The latter organism was entirely absent from guinea pigs fed the basal cereal diet. B. subtilis has been isolated from our active alfalfa meal and alfalfa is known to contain a substance(s) that stimulates the growth of this organism. Studies are in progress to determine the relationship of this organism to enhanced radiation resistance of the guinea pig as a separate phenomenon from improved growth elicited by alfalfa meal but not by cold-water-soluble extract.

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RH 00390
GM
(formerly
RH 00163)

"EFFECTS OF AGING AND RADIATION OF HAARSCHEIBE PIGMENT"

A study on the origin and fate of Haarscheiben in haired, hairless (hr hr), and rhino (hr^{rh} hr^{rh}) mice has been undertaken, and preliminary information is reported in the following publications:

Forbes, P. Donald. Experimentally-induced neoplasms in the skin of mice. I. Single-dose effects of DMBA in haired, hairless, and rhino mice.

II. The influence of age on tumor induction in rhino mice. J. Invest. Derm. June issue, 1965.

Forbes, P. Donald, and Frederick Urbach. Ultraviolet radiation-induced erythema and neoplasia in the skin of mice. Rad. Res. 25: abstr. #48 1965

Both chemical irritants and radiation exposure are shown to be effective modifiers of skin pigmentation. The work is proceeding with embryological studies on the mice named, as well as on alligator (ar ar) mice. The alligator mutant has normal-appearing hair bulbs in which a failure in keratin metabolism prevents the

RH 00390 (continued)

development of a hair shaft. Such animals have hair growth waves, put produce almost no hair above the skin surface.

The possible influence of hair cycles on the expression of Haarscheibe morphology is being investigated. Experimental subjects are haired and alligator mice.

Radiation sources, e. g., 10 KVP, 100 KVP, and 280 KVP X ray, ultraviolet (several wavelengths), and alpha beam (Brookhaven 60" cyclotron) have been used in a study on influencing skin pigmentation, including the Haarscheiben. Preliminary experiments have shown that the alpha particles (as compared with X ray) possess an RBE in the skin of approximately 2 to 6, depending on the circumstances of exposure and parameters of measurement. The third publication listed above reports some of the preliminary UVR work.

Pigment mutants in several strains of mice are being studied in a program which has yielded quantitative data on hair melanin content. A Hunter Color Difference Reflectance Spectrometer makes possible the determination of color in several parameters. The native and induced skin pigment of the same mutants will be studied, with particular attention given to Haarscheibe pigmentation.

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RH 00392
BBC
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RH 00262)

"EFFECT OF RADIATION ON PROTEINS AND RELATED COMPOUNDS"

I The electron spin resonance (ESR) spectra of a number of potential protective agents are being investigated. These are CySSCy, ($Cy{=}CH_2CH(NH_3^+)COO^-$), CYSH, CySSCy, CySSOCy, $CySSCH_2CH_2NH_2$, $CYSSSCH_2CH_2COOH$, $CySSO_3H$, $CySSC(CH_3)_2CH(NH_3)^+COO^-$ and $NH_2CH_2CH(R)SH$ ($R{=}CH_3$, C_2H_5, C_3H_7). The compounds have been prepared, or have been obtained, and their spectral properties are being determined before and after irradiation. Spectroscopic measurements are carried out at room temperature, at 77°K, and at intermediate temperatures. The radiation sources used are

RH 00392 (continued)

both ultraviolet light sources and various forms of ionizing radiation. Some of the compounds have also been converted into their deuterated analogues, in order to assist in characterizing the radicals which give the ESR spectra. Attempts have been made to obtain the substances in the form of single crystals. It is hoped to find correlations between protective ability and some of the features of the ESR spectra.

II ESR spectra and other physico-chemical properties of a number of proteins are being investigated. These properties are being determined before and after irradiation, and the changes observed are compared with the changes observed in the same protein material obtained from animals of different age groups. The purpose of the investigation is to determine the relations, if any, between the effects of aging, and the effects of radiation. The proteins chosed are eye lens proteins which are fairly readily obtained from rats, and which are relatively simple proteins as judged from molecular weight and amino acid analyses. Moreover, they show pronounced changes on irradiation and on aging. They can be separated into different fractions (insoluble and three soluble fractions, α, β and γ, of different molecular weights) using column chromatography. The physical methods used, apart from ESR spectroscopy, are SH-determinations using methyl mercuric iodide, ultraviolet spectroscopy, and as more material becomes available, tritium-hydrogen exchange experiments will be carried out. It is also intended to feed protective agents to animals and note whether this leads to any differences of the observed changes in the properties of the eye lens proteins obtained from irradiated animals.

III The ESR spectra and other physico-chemical properties of human age pigments (heart, liver) are being investigated. The pigments are provided by Dr. D. D. Hendley; age pigments slowly accumulate in the non-dividing cells of the heart and other organs on aging. They could be formed, partly, as a result of prolonged exposure to radiation. The age pigments investigated, which may be composed of more than one type of signal, afford strong ESR signals and the signals thus observed are compared with signals obtained from other polymeric compounds, with or without irradiation, using standard methods of ESR spectroscopy. Work is also in progress to determine other physical and chemical properties of the age pigments, such as their amino acid composition, and their infrared and ultraviolet spectral properties.

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II. MEASUREMENT OF RADIATION EXPOSURE

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RH 00045
RAD

"CLINICAL DOSIMETRY FOR COLBALT-60 THERAPY"

Although occasional measurements are made in the patient during radiation treatment most treatment planning and calculation of dosage is based on standard isodose curves which were constructed from measurements in water or unit density material. These methods lead to two major difficulties. First, when used for arcing or rotation therapy, the calculations are long and tedious, even if simplifying assumptions are made which introduce some inaccuracy. Secondly, errors introduced by the presence of bone and lung in the treated volume are well known, but their magnitude in the patient is uncertain. The actual dose distribution may be considerably different from the calculated one.

The simplest solution to the determination of dose distribution in a complicated treatment pattern may be to expose a film in a simulated human phantom and measure film density with a recording densitometer. In the supervoltage and Co^{60} range this has been done successfully by other workers, but the errors due to energy dependence of the film emulsion, particularly in the depth and at the margins of the field, non-linearity of density with exposure and distortion during development need further investigation.

Current film dosimetry work involves assessment of fixed field dose-density relationship error in both alderson phantom stock and untempered masonite with comparison of both materials to water. In spite of close control of exposure and development factors, film to film sensitivity variation, even within a given batch, seems to place the most serious restriction on the accuracy of parallel film technique. At this stage it appears unlikely that any film dosimetry technique can be suitably accurate without multiple ion chamber correlation of density to exposure.

By comparison of film density to measured exposure at many depths along the central axis and at several levels perpendicular to the axis, assessment is being made of non-linearity of film response. Under certain field size conditions, both over and under response relative to ion chamber have been found. Magnitude and direction of this non-linearity is of course dependent on depth and position in the field.

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RH 00054
(Revised)
RAD

"BACKGROUND RADIATION IN FLORIDA"

Since 1958 the State Board of Health has been measuring radiation levels in water, air, and food and external gamma radiation. Efforts were initially directed at the region encompassed by the Bone Valley phosphate deposit with supplementary data collected in other areas. To date approximately 2100 gamma radiation measurements have been made and 600 air samples, 25 water samples, and 90 food samples collected for the study.

Extensive external gamma radiation determinations were made in northeast Florida, central Florida and northern Jackson County. It appears that Florida, except for the phosphate region and over limited pockets of heavy mineral sands, is characterized by low external gamma radiation levels. However, though the radiation levels over earth appear to be low, measurements made over sidewalks and roads in cities of the phosphate region are much higher. Here levels were recorded between 20 uR/hr and 30uR/hr. The origin of these high street-level radiation levels is currently being investigated.

Average 8:00 a.m. and 3:00 p.m. airborne Radon-222 levels in Bartow, a city in the heart of the phosphate region, were much higher than levels in Orlando and Jacksonville. Radon-220 levels were approximately 1-2 pc/m^3 in Bartow and Orlando; these were higher than Jacksonville. However, the Jacksonville sampler was not as close to the ground. A recording ratemeter system utilizing a G-M detector connected to a chart recorder which continuously monitors a glass-fibre filter through which air is drawn, has shown that nighttime Radon-222 concentrations in Bartow will rise to 2000 pc/m^3 and even 3000 pc/m^3 during the summer. It is estimated that peak levels in Jacksonville and Orlando are about 1200 pc/m^3 to 2000 pc/m^3. Estimates of alpha dose from daughters of Radon-222 to the lower respiratory tract are being made.

Gross alpha content in vegetables grown in the phosphate region was significantly higher than in vegetables grown outside the region. Gross beta content was nearly the same, though also higher in the phosphate-grown vegetables.

Examination of nearly 280 raw well water samples shows a trend toward higher gross alpha content in shallow well water from the phosphate

RH 00054 (continued)

region. This data on shallow well water is still incomplete and no definite statement about the significance of our finding is possible at this time.

Measurements of radiation levels in food, water, air, and external gamma radiation have shown that those persons residing in the region encompassed by the Bone Valley phosphate deposit are being exposed to a higher level of background radiation than persons outside the region. Further measurements of external gamma radiation and airborne radioactivity and qualitative-quantitative analyses made of radionculides in food and water are needed before any estimate of total radiation exposure may be made.

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RH 00084
RAD

"RADIATION EXPOSURE TO GONADS DURING DIAGNOSTIC X-RAY PROCEDURES"

This project was designed to investigate radiation exposure to the gonads during diagnostic x-ray procedures. Initially the principal efforts were devoted to the measurement of testicular radiation exposure incident to the various urographic procedures which from previous experience were known to be associated with relatively high radiation levels.

Radiation fields surrounding urologic x-ray equipment were assessed with reference to the effect of collimation and filtration of the x-ray beam. The value of testicular shielding during clinical urologic examinations was determined by dosimetric analysis. As the opportunity presented itself to study gonadal radiation exposure incident to dermatologic x-ray therapy, an investigation was carried out to measure gonadal exposure during various types of x-ray treatments with special emphasis on site of treatment, limitation of radiation beam, and lead shielding of body and gonads.

RH 00084 (continued)

Early in these studies it became apparent that in order to carry out valid dosimetric determinations, more effort had to be spent on the evaluation of the quality of the scattered radiation originating from the primary x-ray beam. This led to investigation to improve the techniques of phantom dosimetry, and to perform spectral analysis of the scattered x-rays. These studies necessitated the construction of a special scintillation counter and collimators for the determination of the low-energy scattered x-rays. The data thus obtained was analyzed and corrected for the sodium-iodide scintillator escape peak and scintillation detector resolution by computer means.

As the result of these investigations and those of others, it became evident that there was a paucity of information concerning fluoroscopic gonadal exposure. Since the data indicated that relatively large gonadal radiation exposure was due to fluoroscopic examinations, it was elected to pursue studies along these lines in a more limited way. The design of a fluoroscopy simulator and the measurement of gonadal radiation exposure incident to fluoroscopic examinations were continued under research programs RH 00003 and RH 00357.

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RH 00159
RAD

"PHOTOCONDUCTIVE ABSORBED RADIATION DOSIMETRY"

The photoconductivity of a number of materials has been utilized as the basis for dosimetric measurements. Materials such as CdS having an atomic number much higher than any tissue of interest respond well but show an expected photon energy dependent response in the x-ray range which is annoying. This project is devoted to the investigation of photoconductive properties of low atomic numbered materials such as might show energy absorption characteristics not too dissimilar from those of tissue so that they might be applicable to absorbed radiation dose measurements.

RH 00159 (continued)

Whether these should be successful as dosimetric devices depends upon five conditions: (1) the "effective" atomic number of the material should be equivalent to tissue, (2) photocurrents due to externally applied radiation should be linearly dependent upon the energy absorbed in the volume of the material, (3) the material should normally have extremely low conductivity, (4) photocurrents at clinical absorbed dose levels should be sufficient for reliable measurement and (5) the response should not be dependent on exposure or age of the detector.

Anthracene crystals are the current subject of investigation. Photocurrents varying from 2×10^{-12} to 50×10^{-12} have been measured in wafers exposed to radiation having "effective monochromatic energies" from 30 to 100 keV at levels from 1 to 25 roentgens/min and have shown linear and absolute absorbed-dose characteristics.

Some anomalies in residual currents and polarization effect have appeared and are currently under investigation. A study of the basic processes responsible for the observed currents is in progress. The possibility that currents are surface-derived must be considered. There is interest in whether the currents are developed as a result of the formation of charge carriers directly, by x-ray energy photons or indirectly, by the near ultra-violet fluorescent radiation which appears in the crystals under radiation. Currently measurements are under way to seek to supply answers to these questions.

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RH 00316
RAD

"NATURAL RADIOACTIVITY IN HUMANS AND THEIR ENVIRONMENT"

The radioactivity of human body and its environment has become a matter of increasing importance, since the hazards now known to be associated with the intake of small amount of radioactivity from the environment. From this point of view, Misasa, one of the highest radioactive hot springs area in Japan, will offer many opportunities to investigate the distribution of naturally occurring radionuclides

RH 00316 (continued)

of uranium and thorium series in the human environment, mode of entrence of the radionuclides into the human body and the body burden of these radionuclides.

The preliminary study has been conducted according to the following outlines:

1) Investigation on the distribution of naturally occurring radionuclides (uranium, radium, polonium-210 and thorium-228 etc.) in drinking water and food stuffs in the high radioactive hot springs area and in the control area (Okayama)
2) Investigation of naturally occurring radionuclides in human specimens (especially teeth) from life- or long-time residents of the areas.

Water samples from tap water, well water, river water and hot spring water have been collected. Food stuffs including leafy vegetables, fruits, root crops, rice, meat and milk were also collected, in order to obtain the information on the levels of the radionuclides in diet, which consumed by the residents in the areas. Teeth samples of more than 1,500 have also been collected from the residents in the age range from 5 years to 80 years with individual histories, to estimate the levels of the radionuclides in skeletal tissues.

The measurement of uranium was carried out by the fluorometric method. Radium and polonium-210 were analyzed by the methods of Radford et al. Thorium-228 measurement is carried out by the gamma spectorometry, by measuring the thallium-208 peak at 2.62Mev.

Uranium and radium contents in tap water samples from the hot springs area were generally found to be higher than the samples from the control area. Radium content in a hot spring water, which usually used for therapeutic purpose, was observed to be about 21 pc/l. Polonium-210 and radium content in the teeth samples from individuals of 36 to 40 years old, who have resided more than 15 years in the hot springs area and the control area, were also analyzed. No significant difference of radium content between the samples from the both areas was observed, but polonium-210 content in the samples from the hot springs area seemed to be higher than the control samples.

Further studies are in progress on the estimation of dietary intake of naturally occurring radionuclides by the residents of the area, and on the distribution of the nuclides in human specimens of various age groups.

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RH 00342
NTN

"RADIOISOTOPES IN THE TOTAL DIET"

This study is a continuationnof previous Consumers Union studies, started in 1959, of the radionuclide content of the total diet in the U.S. Previous studies, which were assisted by AEC and PHS funds, concentrated on diets of teenagers, but also included some adult and one-year-old infant diets. The present study includes diets of teenagers, pregnant women in the third trimester, and one-month-old infants; the latter two were chosen to furnish data which should be useful in interpreting the findings of the various baby tooth surveys in progress.

Samples are prepared in selected months for the purpose of establishing the dietary levels of these radioisotopes, and to determine the temporal trends and the peak periods. Samplings were made in August and November 1964, and in February 1965.

The samples are prepared by home economists in the cities selected for the study, using locally-purchased foods and local water. The menus are prepared on the basis of surveys of local eating patterns. The foods are prepared by normal procedures as for eating, and, after removal of inedible portions (bones, fat, etc.), are composited. Each composite sample is comprised of 21 meals (one week) and snacks.

Determinations of strontium-90, cesium-137, and calcium are made on aliquot portions after homogenizing the samples in an industrial-size Waring blendor.

The special interest in the teenage diet is due to the fact that this age group builds the largest absolute amount of bone. The infant group has the largest percentage increase in bone. The interest in the diet of pregnant women is due to its influence on the radioisotope intake of the fetus, which is building teeth and bones in the prenatal state, when sensitivity to radiation is greatest.

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RH 00343
DEN

"STRONTIUM 90 EFFECTS ON PULP AND DEVELOPING TEETH"

The purpose of this project is to study the effect of Sr90 on the pulp tissues of rat molars.

As a preliminary study, five groups of 19-day-old Sprague-Dowley rats were injected intraperitoneally with solutions of Sr90 with concentrations of 50, 100, 200, 400, and 600 microcuries per kilogram of body weight respectively. This particular age was selected to correspond to the completion of dentinal formation of the lower first molar crown permitting the Sr90 to be located as close to the pulp tissues as possible. Radiation effects on pulp tissue not actively forming dentin could thus be studied. For the second and third molars where dentinal formation was not complete, the effect of the radiation source on dentinogenesis could thus be assessed.

Both experimental and control animals from each group were sacrificed serially at 6,12,18, 24, and 30 day intervals after injection. Plano-parallel undecalcified ground sections of about 100 u in thickness were prepared from the left mandible after imbedding the specimens in Ward's bioplastic. Histological serial sections, stained with H and E, were prepared from the right mandible.

Autoradiographs were made from each ground section using Kodak Spectrographic plates #649-0. A method for superimposing the autoradiograph and the ground section was developed for the purpose of visualizing the location and the distribution of the Sr90 in the different calcified tissues involved.

The histological sections are being used to evaluate the changes in odontoblastic morphology or activity due to the effect of Sr90 at different concentrations.

A constant finding in the study of the histological sections has been a definite change in staining of that dentin formed after the injection time. This may be interpreted as an alteration of the mineralization process.

RH 00343 (continued)

Observations of the autoradiographs show an increased photographic density in those areas where higher odontogenic activity is taking place.

A more detailed and comprehensive study is in progress.

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RH 00357
RAD

"CRITICAL ORGAN EXPOSURE DURING FLUOROSCOPY"

A determination of radiation exposure of the population as the result of medical X-ray examinations depends upon accurate information of radiation received during a variety of radiologic procedures. In the past most studies have been limited to measurements of radiation exposure to critical organs and selected body segments in radiographic examinations in which radiation factors such as quality and quantity of radiation as well as field size are relatively fixed and not subject to wide variations.

Relatively few studies have concerned themselves with fluoroscopic radiation exposure which is much more difficult to determine because of continuous variations in field sizes and rapid and frequent changes in the position of the X-ray beam in relation to the human body.

The present project has approached the solution of this problem by the development of special fluoroscopic recording and simulating equipment.

During actual clinical radiologic examinations a continuous and permanent record of X-ray tube position, beam area, patient position, and exposure time is made on punch paper tape utilizing the recording system developed in this laboratory.

RH 00357 (continued)

The patient identification, age, weight and height, type of examination, physician identification, and X-ray machine parameters are recorded by an attending technician. The resulting data is evaluated in two ways:

1. A punched paper tape record produced by the recorder is used to control a fluoroscopic simulator developed specifically for this application. The simulator duplicates the original fluoroscopic examination as performed by the radiologist. A tissue-equivalent phantom placed on the simulator table is exposed to the X-ray beam in the same way as the patient was during the original examination. Radiation exposure to selected body segments and critical organs, such as ovaries, bone-marrow and spleen is then measured with small tissue-equivalent ionization chambers placed within the phantom to provide determinations of radiation dose to critical organs.

2. The basic data is also processed by a UNIVAC Solid-State 90 computer. From the recorded paper tape of each examination the computer is programmed to calculate and print out the following:

A. The average surface exposure area.
B. The total exposure time.
C. A topo-anatomical graphic display exposure pattern.
D. The distribution of exposed areas.
E. The integrated surface exposure. This term is a quantity which expresses the total X-ray energy imparted to the patient. It is computed by multiplying the exposure area for each one-second time interval by the X-ray machine output (in roentgens per second) of the fluoroscopic examination. The surface exposure interval has the units of roentgens square centimeters. From the determined surface exposure integral the integral dose can be calculated.

The above results and the critical organ exposure from the simulation measurements are recorded on a single standard punched card with the patient identification, physical characteristics, and type of examination. These cards are used by the computer for tabulation andccorrelation of the various exposure parameters for each kind of examination.

Approximately 1000 fluoroscopic examinations performed by fifteen (15) different doctors with various levels of training and experience at Emory University Hospital are under study.

RH 00357 (continued)

Through graphic representation of the recorded beam area and exposure time for each second of the fluoroscopic procedure, it is possible to make useful comparisons of the techniques used by radiologists in various levels of training when performing similar fluoroscopic examinations. These data also provide comparisons of the exposures resulting from the use of different X-ray technique factors such as occur with the use of image amplification.

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RH 00366
RAD

"ENVIRONMENTAL RADIOACTIVITY"

Accurate measurement of the exposure of man to natural radioactivity and cosmic rays will furnish basic information regarding the radiation health problems of the population.

In the past, extensive measurements have been made of cosmic radiation and background gamma radiation, e.g., with thick-walled ionization chambers. To a much lesser extent, alpha and beta particle radiation have been studied. In laboratories where the total background radiation has been investigated, this has been done through a series of individual, discrete measurements of the various contributors to environmental radiation at different times. Each measurement in turn referred back to previous measurements of estimates to arrive at the resulting values.

The purpose of this project is to develop suitable instrumentation for the simultaneous observation of the basic components of natural environmental radioactivity in order to provide complete data on man's background radiation dosage. Ideally, what is sought, is an absolute determination of the alpha, beta and gamma radiation background in which man lives. The simultaneous variations in the natural and man-made radiation components will be investigated and correlations sought with time and meteorological conditions. The equipment should be of value in differentiating the separate components of natural radioactivity, and so make possible the better assessment of low-level

RH 00366 (continued)

and long-term radiation dosages to the public. Further studies of the variations of the components of environmental radiation with altitude and over various terrains are anticipated based upon the results achieved in this project.

The initial phase of this work was to seek suitable materials from which to construct ionization chambers which has minimal or preferably no inherent residual radioactivity. Since the radiation to be measured, particularly the background alpha radiation, is a very low intensity, it is necessary that the walls of the ionization chambers be constructed of a substance with extremely low radioactivity, That is, the alpha emission must be as low as possible, consistent and accurately predictable. Also, it is desirable that the beta and gamma background radiation similarly be very low or non-existent. Standard laboratory instrumentation on hand was tested for such measurements and was found to be too insensitive and unstable. Subsequently, additional apparatus was assembled and installed in a new low-level radiation measurements laboratory. Many materials were tested, and a preliminary report of the results, as well as a description of the measuring instruments, has been published in THE NATURAL RADIATION ENVIRONMENT, edited by J.A.S. Adams and W.M. Lowder (page 237, the University of Chicago Press, Chicago, 1964).

Experimental ionization chambers have been constructed and are currently being evaluated. Meteorological instruments and data recording apparatus have been secured and are being integrated into the measuring system.

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RH 00404
COM

"DIGITAL COMPUTING TECHNIQUES IN RADIOLOGICAL PROBLEMS"

The subjects of this investigation are those radiological problems amenable to computational analysis, but generally excluding computational techniques in dose planning in radiation therapy.

RH 00404 (continued)

A theoretical analysis has been made of the dose distribution in soft tissue cavities in bone irradiated by X-rays (1), and this has been extended to other non-equilibrium dose distribution problems (2). Comparison of computed dose distributions with experimental measurements are encouraging, but further work is needed and more extensive computations are in progress.

Curve fitting computations have been made to fit analytical expressions to published data on electron (and positron) ranges in a wide variety of materials as a function of energy. These expressions are of value in dose computations in non-equilibrium situations and will be made available shortly through the International Atomic Energy Agency.

In a further study an attempt is being made to construct a mathematical model for the growth and metastasis of malignant tumors in treated and untreated patients and to correlate this with clinical observation.

References

1. J. L. Howarth, Calculation of the absorbed dose in soft tissue cavities in bone irradiated with X-rays. Radiation Research, 24 158-183, 1965.

2. J. L. Howarth, Calculation of the alpha-ray absorbed dose to soft tissue cavities in bone. Brit. J. Radiol., 38: 51-56, 1965

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III. DEVELOPMENT OF METHODOLOGY FOR EXPOSURE REDUCTION AND CONTROL

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RH 00175
NTN

"RADIONUCLIDES IN FLORIDA MILK"

Since the U. S. Public Health Service began Cesium-137 analysis of milk under their Pasteurized Milk Surveillance Network program, above-average values of this radionuclide have been detected consistently in milk in the Tampa, Florida sampling station. Verification of this anomaly with additional supporting data by the Florida Radiological Surveillance Network program prompted further investigation.

A supporting grant was awarded in June, 1964 to fund the proposed investigation of unusual levels, variations in geographical distribution, and causative factors of radionuclides in Florida milk. Implementation of the program was begun in November, 1964 with the general approach to the problem consisting of milk, feed, pasture, and water sampling in the first region of study. Standardization of sampling methodology and laboratory techniques and development of a program for automatic data processing have been initiated.

Preceding program implementation, background information and available data were studied. The Florida Radiation Surveillance Network program began Cesium-137 analysis of regional raw milk sampling of ten per cent of random stratified dairy farms in April, 1963, Due to unusual geological and meteorological conditions and unique dairy practices such as large herds with minimal pasture support, milk production in Florida with high Cesium-137 levels is not readily explainable. While dairy practices and Cesium-137 levels in milk in continental Florida seem to be similar to those reported in Georgia, Alabama, and Louisiana, dairy farm practices in peninsular Florida are unique in that most milk is produced by large herds and forage provides a relatively small percentage of the cows' intake. Although pastures have been given primary attention in explaining differences in Cesium-137 levels in other parts of the country, this factor in Florida would seem to be less important since despite high temperatures and high rainfall, sandy soil does not produce lush forage. Most cows are on pasture all year; however, a processed bulk mix is by far the major dietary intake with hay and silage being an occasional supplement.

Full scale milk sampling (market, plant, farm) was begun in December, 1964 in one of the six regions of the state to be studied. Selected farm sampling of milk, feed, pasture, and water is underway and results of all milk sampling to date indicate a level of Cesium-137 in milk well above the national network average.

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RH 00175 (continued)

The Cesium-137 analyses were determined by gamma spectroscopy from 3.5-liter sample sizes using a 4" X 4" NaI(Tl) crystal detector connected to a 512 channel pulse height analyzer with a counting time of 40 minutes per sample. These data will be programmed and simultaneous equations corrected by computer for interference from other gamma emitting radionuclides using automatic data processing services available.

The only common component of feed sampled to date was citrus pulp and limited sampling of this product indicates a possible area of concentration of Cesium-137. However, more work will have to be done to verify this potential. Pasture varies consistently with White Dutch Clover, Coastal Bermuda, Pensacola Bahia, Carpet and Johnson grasses, none of which is predominant. Water supplies sampled to date were deep wells on all farms with three farms having surface water available for consumption by producing herds. No significant findings of Cesium-137 have occurred in either deep wells or surface water analyses.

Market milk is sampled periodically to relate observations to exposure of the public. Three additional rounds of farm sampling (milk, feed, pasture, and water) and evaluation of findings will be completed in the first region before moving to a second region.

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RH 00276
RAD

"DISTRIBUTION OF RADIOACTIVE PARTICLES IN ENCLOSURES"

The contamination of surfaces with radioactive particulate matter presents a potential hazard to individuals in the environment, particularly through the redispersion and subsequent inhalation and deposition of the material in the lung. Studies are being conducted on the manner in which surfaces become contaminated and on the degree to which radioactive particles may become airborne from surfaces as a function of particle size, radioactivity and nature of the surface.

RH 00276 (continued)

The major effort to date has involved studies of the following problems:

1. Production of radioactive particles:

Barium sulphate particles have been selected for initial studies, as they may be grown under controlled conditions to give particles of the order of 10 microns diameter. The particles may be tagged either with Ba140 or S35. The most reproducible results were obtained when solutions of 5×10^{-4} M barium chloride and 5.07×10^{-4} M sodium sulphate were mixed together in equal volumes at room temperature by dumping one solution into the other and allowing to stand for 24 hours. Uniform crystals of dimensions 15 x 7.5 microns were obtained and were the largest crystals obtained in the studies. The crystals could be readily identified under the microscope and made significantly radioactive with the addition of relatively small amounts of radioactivity.

2. Sampling of the particles and radioautography.

Different emulsions for obtaining radioautographs of the particles were studied. DuPont 508 (now 521) personnel monitoring film was selected because of its sensitivity, availability and ease of handling. Well defined black spots were produced by the particles in less than 24 hours exposure.

Studies of the behavior of these particles require the ability to observe both the particle and its radioautograph. When the particle is sampled or deposited on millipore filter paper, this can be done by making the millipore paper transparent. Best results in clearing the millipore paper were obtained by cementing the millipore to a glass slide with a mixture of collodion-isoamylacetate and clearing with a mixture of one part of dioxane and two parts of petroleum ether. By the use of special frames, the particle and its image could be located to within 15 microns of each other under the microscope.

3. Deposition of particles and dispersion from surfaces:

Suspensions of the particles in water were allowed to dry on plastic and metal surfaces and attempts were then made to remove them by jets of air. It was known that under these conditions, small particles are held rather strongly onto surfaces and that high velocities are required before the air will approach closely enough to the surface and with enough force to remove the particles. However, levels for control of

RH 00276 (continued)

contamination by radioisotope users are set very low, in part because radioactivity on a surface is considered to be a potential airborne hazard if the air around the contamination is agitated. Although air was blown against the contaminated surfaces for many hours, and at speeds of the order of 40 meters per second, very few particles were removed.

Current effort:

Different methods of deposition and resuspension of particles are being tried. The radioactive tag enables ready location of the particles in enclosures. The purpose of this work is to provide insight into the hazard from radioactive contamination, and to help assess the reasonableness of the present extremely low levels used by the regulatory agencies for radioactive contamination in uncontrolled areas.

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RH 00307
RAD

"BIOLOGICAL ENRICHMENT CHAINS OF RADIONUCLIDES IN FINNISH LAKES"

The purpose of this investigation is to elucidate the dependence of the radionuclide contents of the biota of Finnish lakes upon the limnological factors present in the respective lakes.

Fourteen lakes were chosen for the survey. They represent the eutrophic type (rich in nutrients), the oligotrophic type (deficient in nutrients) and types between the two extremes. Some of the lakes are of the humus containing "dystrophic" type, typical in Finland.

The limnologiaal type of the lake was determined by

a) w a t e r a n a l y s e s: oxygen content, conductivity, $KMnO_4$-consumption, colour, visibility and pH, and

RH 00307 (continued)

b) by a qualitative b i o l o g i c a l s t u d y of the biota: determination of the plant and animal species present, their approximate frequency, their food and

c) by a more quantitative estimation of the d i e t o f t h e f i s h by stomach analyses.

From the lakes investigated the following samples are taken:

water, plankton, water plants (10 to 12 "indicator species", if typical), bottom animals and fish (2 to 10 species, if typical).

Of the bottom animal and plankton samples the species and their percentage in the sample is determined.

γ -spectrum of the dried sample is determined by a multichannel analyzer and Cs^{137} and potassium and in some cases Ru^{106}-Rh^{106}, Zr^{95}-Nb^{95}, Fe^{55} and Mn^{54} are analyzed. Total β-activity of each sample is determined after ashing at 400°.

In many of the samples (all of the water samples) a number of inactive elements (Na, K, Ca, Sr, Mg, Fe, Mn, Zn) are determined by atomic absorption or flame spectrometry or by activation analysis to understand their possible roles in the food chains of the above-mentioned fission nuclides in lakes of widely different types.

It has been clearly domonstrated that ^{137}Cs- c o n t e n t o f t h e b i o t a i s g e n e r a l l y i n n e g a t i v e c o r r e l a t i o n w i t h t h e f i r s t g r o u p c a t i o n c o n c e n t r a t i o n i n t h e w a t e r. The present results do not yet allow generalizations to be made regarding the factors effecting the uptake of the other radionuclides.

For a more quantitative analysis of the ^{137}Cs chain, laboratory experiments were carried out with fish of different species and ages for the determination of the b i o l o g i c a l h a l f - t i m e of this nuclide in the fish. Rather long biological half-times (several months) for Cs^{137} in perch were obtained in these experiments. A technique for "whole body" c o u n t i n g o f l i v e f i s h was developed therefore, and experiments are continued in the field.

Fish of 3 species (perch, roach and trout) and of different ages have been labelled by giving an oral dose of Cs^{137}. They are grown in large cages in natural waters and counted at intervals of 3 hrs - 2 weeks to determine the biological half-time of the radionuclide in the fish.

RH 00307 (continued)

It is hoped that by analyzing the diet and estimating the food consumption of the fish, and by determining continuously the radioactivity of the fish, its food and the water they live in, and by knowing the biological half-time of the nuclide studied in the fish, a quantitative balance of the hydrospheric food chain could be determined.

***

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RH 00322
ESE

"SORPTION OF HEAVY METAL RADIONUCLIDES FROM AQUEOUS SOLUTIONS ONTO MINERAL SURFACES"

The purpose of this investigation is to study the sorption of aqueous solutions of heavy metal radionuclides onto mineral surfaces. The radionuclides initially investigated was protactinium-233 a beta and gamma emittor with a half-life of 27 days, decaying to uranium-233, the mineral surfaces being silica of various configurations. The equilibria and rates of sorption were studied, particularly as affected by such variables as pH, temperature, ionic strength, ionic composition, mineral particle size, and liquid flow rate past the mineral solution interface. The rate and reversibility experiments are particularly germane to the question of the fate of the radionuclide in the aquatic environment. Under what conditions and how fast will it be sorbed by minerals in natural water; to what extent and under what conditions will it be released?

Although considerable effort has been devoted to measuring the sorption of radionuclides by suspended and bottom sediments of streams, as well as by soils and specific minerals, their interaction with quartz (or silica) has been essentially neglected. The ion-exchange phenomena associated with the clay minerals have been studied particularly intensely because of their high exchange capacity. Nevertheless, for rivers with abundant sands, the latter have been found to be responsible for as much as 90 percent of the bed material exchange capacity. On the average quartz comprises 30 percent of shale and granite, and 70 percent of sandstone. In a study of the River Nitelva in Norway, it was found that post-glacial clay containing about 30 percent quartz is the chief factor in determining turbidity of the river water.

RH 00322 (continued)

Protactinium is of interest because it is found naturally as protactinium 231, resulting from the decay of uranium-235, and is also present as protactinium-233 in reactor-cooling water. It has been shown that sea water is deficient in concentration of protactinium-231 compared to the amount expected from the radioactive decay of uranium-235, less than three precent of the expected value of protactinium being found. It was also observed from an analysis of the cores of ocean sediments that approximately 100 times as much protactinium-231 was present compared to that expected to be in equilibrium with the uranium. The mechanism for the removal of the protactinium from the sea water into the sediments was not explained.

Three kinds of silica are being employed in this study: porous silica discs, thin silica films, and powdered silica. The purpose of the porous disc experiments is to provide a large surface area so that measureable amounts of protactinium are sorbed from solution, the reduction of the beta count in the latter being used as the measure of the extent of sorption. These discs are principally used in measuring the sorption equilibria.

In order to study the rates of sorption during the fairly short times required for equilibrium to be reached, a lucite cell was fabricated so as to permit an in situ measurement. The gieger tube measures the beta radiation coming from the protactinium in the solution as well as that sorbed onto the upper silica surface in contact with the solution. As the sorption process takes place on this surface, increasing amounts of protactinium are located closer to the geiger tube which then registers a higher beta count. The 0.003 inch silica film permits the beta rays to pass. The magnetic plastic coated stirring bar is rotated at a variable speed by a motor with a rotating magnet placed above the cell. A round paddle attached to the center of stirring bar sweeps solution past the upper silica at variable rates as the bar rotates. This cell permits the sorption to be measured as a function of time without distributing the system, and is particularly useful in controlling the thickness of the unstirred liquid film at the silica-solution interface.

The range of concentration of protactinium-233 initially studied in the porous disc experiments was 5×10^{-11} M to 2×10^{-14} M, the pH varying from 0.9 to 5.2. Subsequent measurements will involve pH values as high as 8 or 9. The ammonium chloride was varied from 0.006 to 1.0 N in order to assess the effect of ionic strength on the sorption process.

RH 00322 (continued)

Of special interest is the possible competition between the uranium and protactinium for sites on the silica surface. In all the experiments the uranium ambient concentration is the higher.

Demonstrated effects of pH, competition of ions for the silica surface, and liquid movement at the mineral solution interface are important factors in the interaction of protactinium and other heavy metal radionuclides with minerals in the aquatic environment.

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RH 00354
NTN

"METABOLISM OF RADIOSTRONTIUM"

The purpose of this study is:

(a) To determine Sr^{89} deposition in the avian egg.

(b) To determine Sr^{89} uptake by the embryo and its effect on embryonic development.

(c) To determine the length of time required to minimize strontium levels in body tissues and to determine the relationship of various mineral supplements in the diet to the body depletion of strontium.

(d) To determine the effects of exposure of animals to Sr^{90} over a long period.

Mature birds have been placed in individual laying cages and injected once each week with Sr^{89} at levels of 10 and 20 microcuries of strontium. The calcium and phosphorus levels employed in these diets are those recommended by the National Research Council. Eggs are being collected and will be collected continually for assay of strontium. Birds are being artificially inseminated in order to obtain fertile eggs for the study of embryonic development.

RH 00354 (continued)

All chicks from birds injected with Sr^{90} are being maintained on various types of experimental diets in order to determine the effects of inorganic constitutients on the excretion rate of Sr^{90}.

At the conclusion of one of the present studies the quantitative transfer of strontium from the dam to the developing embryo and subsequent progeny will be determined.

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RH 00369
ESE

"RADIOACTIVITY IN BOTTOM SEDIMENTS"

The correlation of radiation exposure hazard and radionuclide concentration in the aquatic habitat requires determination of the specific radionuclide's role in the aquatic environment. Previous work in this area has shown that when Cs^{137} is wasted to a natural stream it is concentrated to a high degree in the bottom sediments of the stream.(1) Laboratory investigation has shown that Cs^{137} is sorbed rapidly and selectively by illite clay mineral. The extent and rate of the Cs^{137} illite sorption reaction is sufficient to warrent its application as a "scavenger" for decontamination of low level fuel process reactor waste.(2)

The objectives of the research program described herein is to determine:

1. The effects of aquatic biota (principally bacteria and related forms) on clay-sorbed radioactive fission products,

2. the biological availability of the clay-sorbed radioactive fission products.

Preliminary laboratory investigations and observations have shown small concentrations of clay minerals in water evidenced hydrolysis and formed very stable colloidal solutions. Consideration of these conditions together with the complex nature of clay mineral structure, has prompted development of methods to determine which segment of clay

RH 00369 (continued)

structure (and associated chemistry) determines its behavior in solution. One method which appears to hold promise and which is currently being studied is a neutron activation method. Basically, this method consists of activating a sample of clay mineral by exposure to a high thermal neutron flux. Study of the gamma spectrum of the neutron activated samples by means of multichannel gamma spectrometer has shown that several gamma emitting radionuclides are produced.

The results of the neutron activation study have been rewarding and suggest, with reservations, that this method might be used to quantitatively detail clay sorption reactions as well as biochemical reactions of clay materials, in aqueous solution. Additional experiments are planned to determine the specific radionuclide spectrum of the neutron activated clay as well as the nature and extent of structural damage introduced in the clay mineral by neutron activation.

Previous study by other investigators has shown that illite-sorbed-Cs^{137} is available for plant uptake, however, the uptake rate is rather slow.(3) This finding suggests that perhaps the same situation or result is applicable in an aquatic habitat. The determination of this possible mode of transfer requires basic information regarding the relationship of bacterialand clay minerals in solution. It is not practical to consider a comprehensive study of this area; however, it is appropriate to consider study of bacterial groups on the basis of large differences in colonization, ozygen requirements, extracellular enzymes and forms common to the sediment-water interface environment. A tentative laboratory study of the above mentioned bacterial groups in contact with clay minerals has been initiated and growth response patterns for typical members of the above groups are being developed using batch and chemostat culture techniques.

The basic physical reactions of clay minerals in dilute solutions such as attrition and separation or partition coefficients provided by various mixing techniques have been investigated using illite and Cs^{137}. These studies have shown that for the most part attrition is of small significance irrespective of the method or rate of mixing. The separation of the solid and liquid phases of the clay water mixture by conventional centrifuge technique apparently provides a rapid practical method with good reproducibility. Additional evaluation of this procedure is planned.

(1) T. Tamura and D. G. Jacobs, Health Phys. 2, 391 (1960)

RH 00369 (continued)

(2) E. G. Struxness et al., Health Physics Division Annual Progress Report - July 31, 1960, ORNL - 2994

(3) T. Tamura and J. Murdock, Health Physics Division Annual Progress Report - July 31, 1961, ORNL - 3189

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